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# Health Assessment Document for Acrylonitrile

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16. ABSTRACT

Acute acrylonitrile intoxication in humans, like many volatile organic compounds, results in irritation of the eyes and nose, weakness, labored breathing, dizziness, impaired judgement, cyanosis, nausea, and convulsions. Unlike many of these other organics, acrylonitrile causes severe burns upon contact with the liquid chemical. Some significant functional disorders of the liver and kidney are observed under subchronic exposure conditions; while chronic exposure results in signs (unthrifty appearance, weight loss and early death) that may be related to the low food and water consumption resulting from the unpleasant taste of acrylonitrile in water. Pathological changes in rats believed to be treatment related include hyperplasia and hyperkeratosis of the squamous cells in the brain and mammary gland hyperplasia in females.

The present data base from human and toxicologic studies provides enough evidence such that the International Agency for Research on Cancer (IARC) has characterized acrylonitrile as an animal carcinogen and a likely human carcinogen. Unlike the animal bioassay data, the human data base does not unequivocally demonstrate a causal association.

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# Health Assessment Document for Acrylonitrile

# Final Report

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# Health Assessment Document for Acrylonitrile

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

# NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

# PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for Agency-wide use. The health assessment document was originally developed at the request of the Office of Air Quality Planning and Standards; however, the scope of the assessment has since been expanded to address multimedia aspects. This assessment will help ensure consistency in the Agency's consideration of the relevant scientific health data associated with acrylonitrile.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

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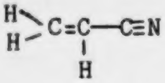
# 1. SUMMARY AND CONCLUSIONS

Acrylonitrile is a clear, colorless, and highly flammable liquid that has an unpleasant and irritating odor. The boiling point of acrylonitrile is 77.3°C; the melting point is -83.55°C, and the density of the liquid at 20°C is 0.8060. Acrylonitrile is soluble in water between 7.2 and 9.1 weight % at temperatures of 0°C and 60°C, respectively. The open cup flash point of acrylonitrile is 0°C, and the explosive limits are between 3.0 and 17% by volume in air at 25°C. Synonyms for acrylonitrile include 2-propenenitrile, cyanoethylene, and vinyl cyanide. Acrylonitrile has a molecular weight of 53.06 and a molecular formula of $C_3H_3N$. The structural formula is given below.



Acrylonitrile monomer production capacity in the United States is approximately 1,128,000 million grams. Of the 862,000 million grams of acrylonitrile produced in 1980, approximately 77% (664,000 Mg) will be used domestically; the remainder is exported. Acrylonitrile is used primarily as a raw material in the synthesis of acrylic and modacrylic fibers, acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile (SAN) resins, adiponitrile, acrylamide, and barrier resins. A small percentage of the acrylonitrile produced is used as a chemical intermediate.

Acrylonitrile is emitted to the atmosphere during monomer production, polymer production, transport, and end-product usage; however, the major sources of acrylonitrile emissions are monomer and polymer production facilities. Of the estimated total of 3,856 Mg emission per year, monomer ABS-SAN resin and acrylic fiber production facilities emit 802 Mg, 1424 Mg, and 1276 Mg, respectively, of acrylonitrile in the atmosphere per year. The atmospheric half-life of acrylonitrile has been estimated to be between 9 and 10 hours (Suta, 1979), which is long enough to allow transport of acrylonitrile from emission sources to nearby populations. In natural waters, acrylonitrile may be removed by either chemical decomposition or microbial degradation (Going _et_ _al_., 1979; Mills and Stack, 1955). Evaporation may [illegible] lower the concentration of acrylonitrile in water; the calculated half-life for acrylonitrile by evaporation alone from water 1 m deep is 795 minutes. The percent of acrylonitrile removal through each of the processes will depend on the characteristics of the aquatic system. For example, in biologically active treatment ponds, less than 0.1% of the acrylonitrile was found to be removed from the aquatic media due to volatilization, while most of the acrylonitrile removal took place via biodegradation.

The acrylonitrile levels in the vicinity of ACN production and polymer manufacturing plants were investigated by Hughes and Horn (1977), Going _et_ _al_. (1979), and Howie (Pedco) (1982).

In general, acrylonitrile levels around user plants may be greater than around producing plants. Acrylonitrile was detected in the air at distances up to 5 km from a user facility; however, the concentrations of acrylonitrile were dependent on meteorological conditions and the production stage within the plant at the time of sampling. No acrylonitrile was detected in the soil near these plants. Variable low levels of acrylonitrile were generally detected in the water downstream from the plants, except for high levels of 35 to 4300 μg/l detected in some samples near wastewater discharge points. Acrylonitrile has also been detected in drinking water, although the levels were not quantified. The inhalation exposure of acrylonitrile in the vicinity of a plant site estimated by dispersion modelling does not agree with the experimental monitoring data obtained from the same site. There are insufficient data with which to determine the human intake of acrylonitrile through food and drinking water.

Limited data suggest that both aerobic and anaerobic microorganisms are capable of degrading acrylonitrile, especially acclimated microorganisms. Certain isolated bacteria can tolerate 10,000 ppm acrylonitrile and use it as a sole source of nitrogen. In natural water, a concentration of 50 ppm may inhibit aerobic microbial degradation of acrylonitrile. The breakdown products of aerobic microorganisms may include ammonia and organics; nitrification of ammonia would follow, producing nitrogen.

Acrylonitrile has been shown to affect some terrestrial and aquatic plants at exposure concentrations of 9 to 100 mg/l. Acrylonitrile is toxic to aquatic animals at exposure concentrations in the low milligrams per liter range. The reported acute LC50 values for fish ranged between 10.1 and 70 mg/l. Subchronic exposure of fish for 30 to 100 days resulted in LC50 values of about 2 mg/l, with no evidence that a threshold concentration had been reached. Although the only tested invertebrate, Daphnia magna, had the lowest acute LC50 value (7.6 mg/l), this species was not adversely affected by chronic exposure to 3.6 mg/l throughout its whole life cycle.

Use of acrylonitrile as a fumigant has shown that the vapor concentrations required to kill 95% or more of many species of pest insects is between about 1 and 10 mg/l. No other information was found concerning the effects of acrylonitrile on wildlife.

Acrylonitrile is readily absorbed in animals following ingestion or inhalation, while dermal absorption is poor and occurs at about 1% of that of the lungs. Following absorption of radiolabeled acrylonitrile, the radioactivity disappears in a biphasic manner, with a half-life for the first phase of 3.5 to 3.8 hours and the second phase of 50 to 77 hours. The predominant route of elimination is through the urine. The routes of elimination are dose-related with the percent eliminated through the urine less for small doses as compared to larger doses, while the relative amount retained by the carcass is greater for the small dose as compared to a larger dose. Acrylonitrile is metabolized to cyanide, which is transformed to thiocyanate and by cyanoethylation of sulfhydryl groups to S-(2-cyanoethyl)cysteine, followed by elimination of these metabolites in the urine. Other minor metabolites are formed from acrylonitrile. The toxicity of acrylonitrile is caused by both the acrylonitrile molecule itself and its metabolites.

Acrylonitrile intoxication in humans results in irritation of the eyes and nose, weakness, labored breathing, dizziness, impaired judgement, cyanosis, nausea, and convulsions. The TLV of acrylonitrile is 4.5 mg/m$^3$ (2 ppm) for humans. Acrylonitrile also causes severe burns to the skin. In experimental animals, there is considerable species variation in susceptibility to acrylonitrile intoxication; the guinea pig is the most resistant and the dog is the most sensitive. In animals, effects of intoxication include respiratory changes, cyanosis, convulsions, and death. In rats, the LD50 for acrylonitrile is between 80 and 113 mg/kg. There is some evidence that acrylonitrile produces abnormal function of both the peripheral and central nervous systems and that acrylonitrile causes damage to the adrenals. With subchronic exposure of animals to acrylonitrile, some signs of functional disorders of the liver and kidney are observed. Chronic exposure of dogs and rats results in unthrifty appearance, weight loss, and early death. Some of these signs may be related to low food and water consumption resulting from the unpleasant taste of acrylonitrile in the water. Pathological changes in the rats believed to be treatment related included hyperplasia and hyperkeratosis of the squamous epithelium of the nonglandular portion of the stomach, proliferation of glial cells in the brain, and mammary gland hyperplasia in females.

Acrylonitrile adversely affected pup survival following exposure of pregnant rats and, in one study, produced teratogenic events. In a three-generation study in which rats were exposed to 500 ppm acrylonitrile in the drinking water, there was reduced pup survival in the first generation. This was a maternal effect inasmuch as fostering the pups on untreated dams eliminated the poor survival. Reproductive capacity was unchanged in the other generations, and the offspring showed no adverse effects on development. Similarly, rats exposed by inhalation to 40 or 80 ppm of acrylonitrile for 6 hours a day on days 6 to 15 of gestation had no statistically significant changes in reproductive success or fetal development. Only the pups of rats administered acrylonitrile *per os* (65 mg/kg) for days 6 to 15 of gestation had an increase in malformations. This increase was in total malformations, with no statistically significant increase occurring in any single malformation. It was concluded that these fetal abnormalities were the result of acrylonitrile and not the result of toxicity in the dams. Although several studies have been conducted to evaluate the ability of acrylonitrile to cause adverse teratogenic, embryotoxic, and reproductive effects, the limitations of the available data do not allow for a full assessment of these effects.

There is evidence that acrylonitrile and an epoxide metabolite causes point mutations in bacterial test systems, and there is suggestive evidence that acrylonitrile may produce a positive response in the sex-linked recessive lethal mutation assay in *Drosophila melanogaster* (Benes and Sram, 1969). Chromosomal damage was not detected in plants or whole animals when treated with acrylonitrile. *In vitro* DNA binding studies indicate that acrylonitrile in the presence of a rat liver activation system as well as its epoxide metabolite, 2-cyanoethylene oxide binds DNA. Acrylonitrile also induces sister chromatid exchange in Chinese hamster ovary (CHO) cells but requires metabolic activation.

From the data base available, it appears that acrylonitrile may have the potential to bind to DNA and cause genetic damage. If the pharmacokinetics of this chemical substance in humans results in metabolic products that can interact with DNA, it also may cause somatic mutations in humans.

Acrylonitrile is not a direct acting carcinogen; hence, the localization and nature of the effects depend on its metabolism. It is probable that the proximal carcinogen is 2-cyanoethylene oxide, since it has been demonstrated as a reaction product with calf thymus DNA. However, the metabolite has not been tested directly for its carcinogenicity. It has been shown to be produced in the liver and possibly circulates to other organs. However, studies have not been done to determine where else in the body this metabolite is produced. There appears to be a clear difference between animals and humans in their tumorigenic response to acrylonitrile: no lung tumors have been produced in animals and no brain tumors have been observed in humans. There are no human studies on the metabolism of acrylonitrile, and there are no pharmacokinetic studies that would be relevant to the characterization of dose-response relationships at low levels of exposure.

The carcinogenicity of acrylonitrile has been studied in seven cancer bioassays in rats: four in drinking water, one by gastric intubation, and two by inhalation. In addition, 10 epidemiologic studies of cancer incidence have been reported. A short description of these studies is presented below.

Quast et al. (1980a) administered acrylonitrile in drinking water to Sprague-Dawley rats for 2 years at dose levels of 35, 100, and 300 ppm. A statistically significant incidence of tumors was observed in the central nervous system, Zymbal gland, stomach, tongue, and small intestine in both male and female rats, as well as in the mammary gland of female rats. The occurrence of central nervous system and Zymbal gland tumors in Sprague-Dawley rats was further confirmed in four other studies: a three-generation reproduction study performed at Litton-Bionetics by Beliles et al. (1980); three studies by Biodynamics Inc. (1980a,b,c) in which acrylonitrile was administered in drinking water and via gastric intubation; and an inhalation study by Quast et al. (1980b).

A second inhalation study by Maltoni et al. (1977) exposed rats to atmosphere containing 5, 10, 20, and 40 ppm acrylonitrile 4 hours/day, 5 days/week, for 12 months. Marginal increases in tumors of the mammary gland in females and the forestomach in males were observed, although the sensitivity of this test was limited by the relatively low dose levels and the short duration of exposure.

Ten epidemiologic studies of the association between acrylonitrile exposure and cancer incidence have been reported: five published (Monson, 1981; O'Berg, 1980; Thiess et al., 1980; Werner and Carter, 1981; Delzell and Monson, 1982) and five unpublished (Gaffey and Strauss, 1981; Herman, 1981; Kiesselbach et al., 1980; Stallard, 1982; and Zack, 1980). Six of these studies present no evidence of carcinogenic risk from exposure to acrylonitrilc. However, all suffer from problems in the design or methodology, (i.e., small cohort size, insufficient characterization of exposure, short follow-up, and relatively youthful cohort). Because of these problems, none of these studies can be cited as adequate evidence that acrylonitrile is not carcinogenic.

Data presented in the remaining four epidemiologic studies consistently demonstrate a statistically significant risk of lung cancer in various subgroups of the populations studied. All four have problems with the methodology, definition, and/or size of the population, whether or not exposure to other carcinogens occurred, and short follow-up intervals. In three of the four studies (Delzell and Monson, 1982; Thiess et al., 1980; Werner and Carter, 1981), the problems were sufficient to cast doubt on the finding of significantly elevated risks of lung cancer reported in each study. In the fourth study by O'Berg, the problems were insufficient to obscure the significant finding of lung cancer. After adjusting for latent factors and evaluating the contribution due to smoking, the finding of a statistically significant elevated risk of lung cancer remained. Thus, one study appears adequate and three are suggestive, while the remaining six are inadequate to address the issue of a risk of lung cancer.

In addition to lung cancer, two other findings of concern are the significantly elevated risk of lymph system cancer found in the Thiess et al. (1981) study (4 observed versus 1.38 expected, $P<0.05$) and the significantly elevated risk of stomach cancer found in the Werner and Carter study (5 observed versus 1.9 expected, $P<0.05$). These findings provide additional suggestive evidence of the carcinogenicity of acrylonitrile.

This level of animal evidence would be regarded as "sufficient" evidence of carcinogenicity according to the International Agency for Research on Cancer (IARC) classification scheme. The human evidence for the carcinogenicity of acrylonitrile would be regarded as somewhere between "sufficient" and "limited," using the IARC classification. Therefore, in combining the human and animal evidence, acrylonitrile would be placed in group 2A, which IARC characterizes as "probably carcinogenic in humans, where the evidence for human carcinogenicity is almost sufficient."

To provide a rough estimate of the potency of acrylonitrile relative to other chemicals and a crude indication of population risks associated with known exposure, unit risk estimates have been calculated. The unit cancer risk for air is defined as the lifetime cancer risks occurring if an individual is exposed to air containing 1 $\mu g/m^3$ continuously for a lifetime. The linear non-threshold dose extrapolation model has been used to give a rough but plausible upper-bound of risk; that is, the true risk is not likely to be higher than the estimate but could be lower. For a discussion of the limitations and uncertainties of the procedure the reader is referred to section 13.5.3.1.

Three unit risk estimates for air are calculated: one based on a human occupational study (O'Berg, 1980a,b) and two based on rat cancer bioassays (Quast et al., 1980a,b). The upper-bound lifetime risk of cancer associated with lifetime inhalation exposure of 1 $\mu g/m^3$ is $6.8 \times 10^{-5}$ from the human study and $1.5 \times 10^{-5}$ from the rat inhalation study. The value based on the rat drinking study is $1.5 \times 10^{-4}$ (or $2.6 \times 10^{-5}$ if the equivalent human dose is assumed to be mg/kg/day rather than surface area) but this study is less reliable because of the inappropriate route of exposure.

The estimate based on the human study is uncertain because of the relatively weak documentation of the available exposure estimates of the acrylonitrile workers. The air concentration had not been measured when the workers experienced their heaviest exposure and was estimated 12 years after the end of the exposure period. However, in spite of these difficulties, the estimates are consistent with those of the animal studies.

The upper-bound risk estimate for 1 $\mu g/\ell$ of acrylonitrile in drinking water is $1.5 \times 10^{-5}$, based on the mean value of three drinking water studies in rats.

There is evidence that acrylonitrile is a human carcinogen. This conclusion is based on 1) findings of three positive drinking water rat bioassays and one positive rat gastric intubation study; 2) statistically significant positive findings of respiratory cancer in four epidemiologic studies; 3) the positive mutagenic evidence in bacteria and sister chromatid exchange tests; 4) in vitro evidence of interaction of acrylonitrile and/or its metabolites with DNA; and 5) acrylonitrile's structural similarity to vinyl chloride, a known animal and human carcinogen.

The carcinogenic potency of acrylonitrile is in the third quartile among 54 suspected carcinogens evaluated by the Carcinogen Assessment Group.

Using the IARC classification scheme, this level of evidence in animals and humans would be considered sufficient for concluding that acrylonitrile is likely to be a human carcinogen with rank of 2A.

## 1.2 RESEARCH NEEDS

The present data base from human and toxicologic studies provides enough evidence such that the International Agency for Research on Cancer (IARC) has characterized acrylonitrile as an animal carcinogen and a likely human carcinogen. Unlike the animal bioassay data, the human data base does not unequivocally demonstrate a causal association. In addition to the human data, there are also limitations in the available animal bioassay data within the areas of reproduction and genotoxicity, which have an important bearing on both the qualitative and quantitative aspects of carcinogenicity.

The highest priority with respect to future recommendations for research should be placed on well-designed epidemiology studies, (i.e., case referent or more vigorously designed historic and prospective studies that will adequately sort out and control the effects of smoking). Careful workplace exposure monitoring should be routinely conducted on members of the study population in order to adequately determine acrylonitrile dosage. In the opinion of some expert epidemiologists, the available cohort identified in the O'Berg study involving DuPont acrylonitrile workers in Camden, S.C. should be followed up as long as possible to determine if a significant elevated risk remains for all site-specific cancers as well as for the higher frequency of lung and intestinal (colon) cancer. In connection with this follow-up study, it would be advantageous to collect information on histopathology of lung and brain tumors to correlate with the reported absence of lung tumors and the presence of brain tumors (glial cell) in animals.

Although several studies have been conducted to evaluate the ability of acrylonitrile to cause adverse teratogenic, embryotoxic and reproductive effects, the limitations of the available data do not allow for a full assessment of these effects. Any new studies that are conducted should be designed to demonstrate the possibility of a dose-response relationship.

Additional carcinogenesis testing is recommended using mice exposed to acrylonitrile by the oral and inhalation routes of administration, because the available animal bioassay data are limited to rats. Acrylonitrile carcinogenesis could also be evaluated using in vitro cell transformation systems, such as BALB/c 3TC cell lines, Syrian hamster embryo cell cultures, rat hepatocyte cell strains, and retrovirus-infected rat embryo cells. An understanding of the mechanism of acrylonitrile carcinogenesis (i.e., genetic versus epigenetic) would be enhanced by pharmacokinetic and macromolecular binding studies in mice and rats. Adequate pharmacokinetic data are presently available only in the rat. In addition, the selection of a model animal species for comparison of the effects of acrylonitrile to humans would be aided by comparative in vitro metabolism studies using target organ homogenates from several species, including humans.

With respect to acrylonitrile mutagenicity, further testing is needed in eucaryotic organisms, other than Drosophila melanogaster, to confirm that acrylonitrile is genotoxic as indicated by the positive response observed in bacteria. An assessment of genetic risk with respect to germ cell mutagenicity cannot presently be made because of the lack of appropriate data.

# 2. INTRODUCTION

EPA's Office of Research and Development has prepared this health assessment to serve as a "source document" for Agency use. This health assessment was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulations of acrylonitrile under Section 112 of the Clean Air Act. However, based on the expressed interest of other agency offices, the scope of this document was expanded to address acrylonitrile in relation to sectors of the environment outside of air. It is fully expected that this document will serve the information needs of many government agencies and private groups that may be involved in decision-making activities related to acrylonitrile.

In the development of the assessment document, existing scientific literature has been surveyed in detail. Key studies have been evaluated and summary and conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified.

The present document represents an up-to-date data base. The document considers all sources of acrylonitrile in the environment, the likelihood for its exposure to humans, and the possible effect on man and lower organisms from absorption. The information found in the document is integrated into a format designed as the basis for performing risk assessments. When appropriate, the authors of the document have attempted to identify gaps in current knowledge that limit risk evaluation capabilities.

# 3. PHYSICAL AND CHEMICAL PROPERTIES

## 3.1 SYNONYMS AND TRADE NAMES

Chemical Abstracts Name: 2-propenenitrile

CAS No.: 107-13-1

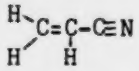
EPA Toxic Substances List No.: R037-2101

RTECS No.: AT52500

Standard Industrial Code: 2822; 2824

The compound is also known as acrylonitrile (AN), cyanoethylene, propenenitrile, and vinyl cyanide (VCN). Fumigant formulations containing acrylonitrile with names Acrylon, Carbacryl, Fumigrain, Ventox, and ENT-54 are no longer manufactured in the United States.

## 3.2 STRUCTURAL AND MOLECULAR FORMULAS AND MOLECULAR WEIGHT



$C_3H_3N$ Molecular Weight: 53.06

## 3.3 BOND ANGLES AND BOND DISTANCES

A molecule of acrylonitrile is planar; all the bond angles are close to 120°. Estimated bond distances are as follows (Wilcox and Goldstein, 1954): C-H: 1.09 Å; C-C: 1.46 Å; C=C: 1.38 Å; and C ≡ N: 1.16 Å.

## 3.4 PHYSICAL PROPERTIES

### 3.4.1 Description

Acrylonitrile is a clear, colorless, and highly flammable liquid that has an unpleasant and irritating characteristic odor (Fassett, 1963).

### 3.4.2 Boiling Point

77.3°C at 1 atmosphere (Groet, 1978).

### 3.4.3 Melting Point

-83.55°C (Groet, 1978).

3.4.4 Density

$d_4^{20}$ (liquid) : 0.8060 g/ml; vapor density: 1.83 (air=1) (Groet, 1978).

3.4.5 Refractive Index

$n_D^{20}$ : 1.3911 (Weast, 1976).

3.4.6 Spectroscopic Data

The $\lambda_{max}$ is at 203 nm with a molar extinction coefficient of 6100; a compilation of infrared, Raman, NMR, and mass spectral data is available in Grasselli and Ritchey (1975).

3.4.7 Solubility

Acrylonitrile is soluble in water, acetone, and benzene (Weast, 1976); miscible with ethanol, carbon tetrachloride, ethyl acetate, ethylene cyanohydrin, liquid carbon dioxide, ether, toluene, petroleum ether, and xylene (Miller and Villaume, 1978).

The solubility in water is given below (Groet, 1978):

| | |
|---|---|
| 0°C: | 7.2 weight% |
| 20°C: | 7.35 weight% |
| 40°C: | 7.9 weight% |
| 60°C: | 9.1 weight% |

3.4.8 Volatility in Water

Henry's law constant: 0.063 at 25°C (Bocek, 1976). Partial vapor pressure (water azeotrope): $\log P = 7.518 - \frac{1644.7}{T(\text{in K})}$ i.e., 80 mm at 20°C (Miller and Villaume, 1978). The half-life of evaporation of acrylonitrile from water with an assumed 1 meter depth can be calculated, using the method of Dilling (1977), to be 795 minutes. The method of Dilling (1977) is applicable for slightly soluble organic compounds present in a static aquatic system. Therefore, the calculated evaporative half-life for moderately soluble acrylonitrile present in a naturally flowing aquatic system may not be accurate.

### 3.4.9 Volatility

Values for the vapor pressure of acrylonitrile (mm of mercury) at different temperatures are given below (Groet, 1978):

8.7°C: 50.3
23.6°C: 99.8
45.5°C: 249.8
64.7°C: 500.3
77.3°C: 759.8

### 3.4.10 Stability

Flash-point (open cup): 0°C (Steere, 1968); Flash-point (closed cup): -1°C (Patterson et al., 1976), -4.4°C (Miller and Villaume, 1978).

Explosive limits: 3.0 to 17% by volume in air at 25°C (Steere, 1968).

Ignition temperature: 481°C (Steere, 1968).

### 3.4.11 Octanol-water Partition Coefficient

k = 0.12 (Leo et al., 1971).

### 3.4.12 Conversion Factor

1 ppm in air = 2.17 mg/m$^3$ at 25°C.

## 3.5 CHEMICAL PROPERTIES

### 3.5.1 Reactivity

Undergoes reactions at both the nitrile group and the double bond (Maltoni et al., 1977). Some of these reactions are used for the quantification of acrylonitrile and have been discussed in Section 4. Acrylonitrile also undergoes the following reactions (Miller and Villaume, 1978), many of which are important commercially.

### 3.5.2 Polymerization

Polymerization, forming high molecular weight products, is the most important commercial reaction of acrylonitrile. The polymerization usually requires the presence of free radical initiators, such as peroxydisulfate. Heat or light ($\lambda < 2900$ Å) can also initiate polymerization reactions. Oxygen and methylhydroquinone are powerful inhibitors of the polymerization reaction.

Pure polyacrylonitrile cannot be dyed using conventional techniques. Copolymerization of acrylonitrile with small amounts of methyl methacrylate or vinyl pyridine introduces reactive dyeing sites. Acrylonitrile can be copolymerized with other monomers as well; examples of other acrylonitrile copolymers include nitrile rubber, acrylonitrile-butadiene-styrene (ABS), and styrene-acrylonitrile (SAN) resins. Terpolymers of acrylonitrile or methacrylonitrile are the so-called barrier resins.

### 3.5.3 Reaction at the Nitrile Groups

Acrylonitrile when reacted with 84.5% $H_2SO_4$ at 100°C produces acrylamide sulfate, which yields acrylamide upon neutralization:

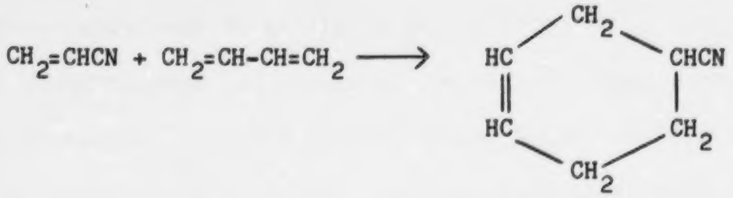
$$CH_2{=}CHCN + H_2O + H_2SO_4 \longrightarrow CH_2{=}CHCONH_2 \cdot H_2SO_4$$

$$CH_2{=}CHCONH_2 \cdot H_2SO_4 + 2NaOH \longrightarrow CH_2{=}CHCONH_2 + Na_2SO_4 + 2H_2O$$

Until recently, the above process was used exclusively for the commercial production of acrylamide. The catalytic hydration of acrylonitrile is the currently used method for the production of acrylamide. When acrylonitrile is heated with less concentrated $H_2SO_4$ or with water, acrylic acid ($CH_2{=}CHCOOH$) is formed. In aqueous NaOH, $NH_3$ is the reaction product. The hydrolysis constant for this reaction was measured as $3.8 \times 10^{-3}$ min$^{-1}$ at 60°C and 1.39 min$^{-1}$ at 100°C (Linetskii and Serebryakov, 1965). Violent polymerization, however, has been reported to occur with concentrated alkali (Steere, 1968). Acrylonitrile allowed to react with alcohols in the presence of concentrated $H_2SO_4$ produces esters of acrylic acid. With olefins, it forms N-substituted acrylamides in the presence of concentrated $H_2SO_4$.

### 3.5.4 Reactions at the Double Bond

The double bond in acrylonitrile acts as a dienophile in the Diels-Alder reaction. Cyclic products are produced when acrylonitrile is treated with aliphatic or alicyclic compounds containing conjugated carbon-to-carbon double bonds. An example is the reaction with butadiene:



Δ-3-tetrahydrobenzonitrile

In the presence of catalyst, acrylonitrile can be hydrogenated to propionitrile, which can be further hydrogenated to n-propylamine:

$$CH_2{=}CHCN \xrightarrow{H_2} CH_3CH_2CN \xrightarrow{H_2} CH_3CH_2CH_2NH_2$$

It can also be reduced, in the presence of magnesium and methanol, in the following manner to produce adiponitrile:

$$2CH_2{=}CHCN + 2CH_3OH + Mg \longrightarrow 2CH_2CH_2CN + (CH_3O)_2Mg$$

This is then further reduced to hexamethylenediamine, which is used in the production of nylon.

### 3.5.5 Cyanoethylation Reactions

These reactions involve the interaction of acrylonitrile with compounds containing active hydrogen. Examples of compounds containing active hydrogen are water, alcohols, ammonia, amines, mercaptans, aldehydes, and inorganic acids and their salts (Miller and Villaume, 1978). The generalized reaction can be written:

$$CH_2{=}CHCN + AH \longrightarrow ACH_2CH_2CN$$

The cyanoethylation of pseudouridine, inosine, and 4-thiouridine by acrylonitrile has been studied as a model for the cyanoethylation of intact tRNA (Miller and Villaume, 1978).

### 3.6 CHARACTERISTICS OF THE CHEMICAL PRODUCT

Technical grade acrylonitrile is a highly purified product with greater than 99% purity. The major impurity is water, which is usually present at a maximum of about 0.5%. The water improves the stability of the product. Other possible trace contaminants include acetone, acetonitrile, acetaldehyde, iron, peroxides, and hydrogen cyanide. Highly pure acrylonitrile may polymerize spontaneously. To prevent this, methylhydroquinone (35-50 ppm) is added to the commercial product. Yellowing upon exposure to light indicates photoalteration to saturated derivatives.

### 3.7 CONCLUSION

Acrylonitrile is moderately soluble in water. Because acrylonitrile does not appreciably dissociate in water, hydrogen cyanide is not expected to be a product of hydrolysis. Because the vapor pressure of acrylonitrile is appreciably high, most atmospheric emissions from its manufacture and use should occur as vapor. The evaporation rate of acrylonitrile from water is appreciable. Therefore, evaporation from contaminated water surfaces can be expected to occur.

# 4. SAMPLING AND ANALYTICAL METHODS

The level of acrylonitrile has been determined in a number of environmental media of interest. These include (1) air; (2) water; (3) waste water; (4) soil and sediment; (5) residue in polymers and the extent of monomer migration in food-simulating solvents; and (6) various other media. All these media can directly or indirectly affect the environmental level or human intake of acrylonitrile.

The sampling method is generally dependent on the medium intended to be monitored. The analysis of samples can be divided into two steps, namely, pretreatment or clean-up procedure(s), when necessary, and quantification procedures. The selection of a particular identification and quantification method is dictated by the accuracy, reproducibility, detection limit, and the possible interference(s) of the method. The sampling and analysis of acrylonitrile in the individual media are discussed below.

## 4.1 AIR

### 4.1.1 Sampling

The collection of acrylonitrile from air has been done by two methods. The first method consists of direct collection of samples without preconcentration. The second method employs the concentration of acrylonitrile in a collection medium during sampling.

In direct sample collection, the air is drawn into plastic bags (Keresztesy et al., 1977) via a two-way valve by means of a sampling pump. The samples are then transported to the laboratory for analysis.

The disadvantage with direct collection is that it does not allow any concentration of the sample and may cause additional problems during transport and storage of samples. Therefore, the detection limit of the method is not satisfactory for ambient air samples, even with the most sensitive method of detection available presently. The advantage of direct collection is that the samples can be analyzed without any pretreatment, thereby reducing analysis time and avoiding any sample losses. It also provides a method for continuous area monitoring of acrylonitrile and for monitoring acrylonitrile from stack and other high concentration emission sources.

In the preconcentration method for sample collection, the air containing acrylonitrile is passed through either a solid sorbent or a trapping liquid medium. Table 4-1 lists the different sorbents and trapping media used for collection of acrylonitrile in air. Activated carbon, silica, and porous polymers have been used as solid sorbents. The sampling unit usually consists of a battery operated pump and a rotometer that indicates the sample flow rate through the sorbent. A tube that contains the solid sorbent is held vertically at a height of 1.5 m from the ground and is connected to the rotometer and the pump unit. Air is drawn through the sorbent tube at a certain flow rate for a specified length of time. At the end of sample collection, the tube is closed with caps and shipped to the laboratory for analysis.

In addition to the error that could be made in measuring the air volume (calibrated pumps may not maintain constant air flow over the entire sampling period), another disadvantage of the solid sorbent method is that sample loss will occur if the breakthrough capacity for the sorbent is exceeded. Therefore, the breakthrough capacity of acrylonitrile through the sorbent should be determined experimentally. The breakthrough capacity is dependent on the relative humidity of air sampled and the presence of other interferences in the air. The concentration of the compound being collected, however, affects the breakthrough volume only slightly. A pollutant present at 20 ppm will break through at only a slightly smaller volume than when present at 1 ppm (Russell, 1975).

Table 4-1. Various Sorbents and Trapping Media for Collection of Acrylonitrile in Air

| Sample | Sorbent | Sampling Condition | Desorption Method | Analysis Technique | Confirmation Technique | Detection Limit | Reference |
|---|---|---|---|---|---|---|---|
| --- | $SiO_2$ | 0.5 lpm[a] for 4-6 l | Water | Colorimetric | None | --- | Krynska, 1970 |
| Plastic producing plant | Silochrom-2 at -50°C | --- | Thermal at 200°C | GC-FID[b] | None | --- | Korzhova et al., 1974 |
| Acrylic fiber factory | Silica gel | Personnel sampler for 100 min. | Water | GC-FID | None | 0.1 ppm | Sakurai et al., 1978 |
| Simulated air | Activated carbon | 15 l in gas bag | 2% acetone in $CS_2$ | GC-FID | None | 0.25-0.5 ppm | Gagnon and Posner, 1979 |
| Plastic processing plant | Activated carbon | 0.2 lpm for 8 hr. | 2% acetone in $CS_2$ | GC-FID | None | 0.03-0.31 ppm | Marrs et al., 1978 |
| Eleven production plants | Activated carbon | 1.0 lpm for ≈24 hr. | $CS_2$ | GC-FID | None | <0.3 μg/m$^3$ | Going et al., 1979 |
| Simulated air | Porapak N | 0.1 lpm for 3-5 l | Thermal at 200°C | GC-FID | None | <1 ppb | Russell, 1975 |
| Simulated air | Porapak N | 0.1-0.15 lpm for 3-5 l | Thermal | GC-FID | None | <0.26 ppm | Campbell and Moore, 1979 |
| Settling pond in acrylonitrile plant | Porous polymer Tenax GC(?) | -0.07 m$^3$/hr. for 5-30 min. | Thermal | GC-FID | None | 10 ng | Hughes and Horn, 1977 |
| Occupational air | Activated carbon | 0.2 lpm for 20 l max | $CH_3OH$ | GC-FID | None | Range 5-135 mg/m$^3$ | NIOSH, 1977a |
| Occupational air | Carbosieve B | 1 lpm for 10-20 l | $CH_3OH$ | GC-FID | None | Range 40-270 mg/m$^3$ | Barrett, 1974 |
| --- | 95% ethanol | 1 lpm | --- | Polarographic | None | 0.5-5 mg/50 ml | Rogaczewska, 1964 |

Table 4-1. Various Sorbents and Trapping Media for Collection of Acrylonitrile in Air (cont.)

| Sample | Sorbent | Sampling Condition | Desorption Method | Analysis Technique | Confirmation Technique | Detection Limit | Reference |
|---|---|---|---|---|---|---|---|
| Grain fumigant | Chilled water | 0.03-0.33 lpm | --- | Polarographic | None | 1.08 x $10^{-5}$ M | Berek, 1962 |
| --- | Water | 0.015 lpm for 1.5-3 l | --- | Colorimetric | None | --- | Nagateva and Nakrap, 1978 |
| --- | Water | --- | --- | Colorimetric | None | 0.5 mg/$m^3$ | Rogaczewska, 1976 |
| Occupational air | Chilled water | 1 lpm for 20-60 l | --- | u.v. | None | Range 10-100 ppm | Brieger et al., 1952 |
| Acrylonitrile plant | Chilled water | 1 lpm | --- | GC-FID | None | 50 ppb | Hughes and 1977 |
| --- | Water | --- | --- | Colormetric | None | 0.5 mg/$m^3$ | Russkikh, 1973 |
| --- | $H_2SO_4$ and glass beads | 1 lpm | --- | Titrimetric | None | 20-300 µg/ml | Gunther and Blinn, 1955 |
| Rubber plant | 1% $H_2SO_4$ | --- | --- | Colormetric | None | 0.85 µg/l | Babanev, 1960 |
| --- | 1% $H_2SO_4$ | --- | --- | Colormetric | None | --- | Arato and Bittera, 1972 |
| SAN Plant | DMF | 0.1-0.3 lpm | --- | GC-FID | None | 0.5 mg/$m^3$ | Babina, 1970 |
| --- | 25% methanol at 15°C | --- | --- | GC-FID | None | --- | Fasitta and Llleciardello, 1977 |

[a] lpm = liters per minute

[b] GC-FID = gas chromatography flame ionization detectors

For air nearly saturated with moisture, the breakthrough volume for acrylonitrile in Porapax N (4" x 1/4" tube) was determined to be 3 to 5 liters. Breakthrough volumes can be increased by using longer sampling tubes. These tubes, however, will result in increasing the back pressure in the tube.

Activated carbon is the most widely used sorbent for the collection of acrylonitrile in air. The adsorption parameters for activated carbon will vary depending on the nature of the carbon and the treatment it received. With 6-10 mesh BC-AC granular carbon, Sansone et al. (1979) determined the adsorption capacity and adsorption rate constant for acrylonitrile to be 0.404 g/g and 116.0 $min.^{-1}$, respectively. With acrylonitrile that contained 50% relative humidity, Nelson and Harder (1974) determined the adsorption capacity to be 0.174 g/g. The breakthrough times for 1%, 10%, and 99% passage from a 58 g charcoal in a respirator cartridge were determined to be 48.5 min., 61.1 min., and 168 min., respectively (Nelson and Harder, 1974). The types of activated carbon used in the above experiments were different.

The effect of humidity on sample recovery by 1.5 g activated carbon was studied in detail by Going et al. (1979). He observed that introducing a Drierite drying tube to absorb moisture did not significantly improve recoveries at relative humidities equal to or exceeding 70%; however, the average recovery for acrylonitrile with air volumes of 750 liters/g or greater remained acceptable at 75 ± 9.4% at relative humidities up to 60%, with or without the drying tube.

NIOSH (1977b) determined that the maximum amounts of acrylonitrile that could be collected with a sorbent tube (8 cm x 5 mm) from 100 ppm acrylonitrile in air were 6 mg, 15 mg, and 22 mg when the relative humidities were greater than 95%, equal to 50%, and less than 5%, respectively.

Segmentation of the carbon tubes into a front and back section remains the most widely used method for the determination of breakthrough capacity limit during sample collection. If the second tube retains more than a certain predetermined percentage of acrylonitrile, it indicates that the breakthrough limit has been exceeded.

It is important to determine the stability of acrylonitrile in the sample tubes during transportation and storage. Marrs et al. (1978) showed that acrylonitrile would remain stable on the carbon tubes at room temperature for a minimum of 5 days. Going et al. (1979) demonstrated that acrylonitrile would remain stable up to 8 days on charcoal tubes stored at -17°C and up to 24 days on tubes stored at -78°C.

The collection of acrylonitrile in air by liquid trapping media has been used rarely in recent years because of the inconvenience in handling and transporting the impingers and the need for cooling the trapping media during sample collection. The collection efficiencies and the storage stabilities of acrylonitrile in these media are not always known. The collection efficiency of chilled water as a trapping medium was determined to be over 98% (Berck, 1962; Brieger, 1952). Virtually no data are available regarding the storage stability of acrylonitrile in this trapping medium, but it is expected to be reasonably stable in water and 1% $H_2SO_4$, particularly since Going et al. (1979) demonstrated that acrylonitrile was stable for about 23 days in neutral distilled water and at a pH of 4. Acrylonitrile collected in aqueous methanol and ethanol and stored at 3°C can be expected to be fairly stable, based on the evidence of Going et al. (1979), who demonstrated that acrylonitrile in carbon disulfide remained stable for over 12 days when stored at 3°C.

### 4.1.2 Analysis

#### 4.1.2.1 Pretreatment

Acrylonitrile collected on solid sorbents requires a desorption procedure before identification and quantification. Thermal desorption and solvent desorption are two commonly used methods. Thermal desorption normally uses gas chromatographic methods for identification and quantification. In this procedure, the sorbent tube is heated between 100°C and 200°C in the injection port of a gas chromatograph. To avoid peak broadening, the position of the sample tube in the gas chromatograph injection port should be in the reverse order as that used during sampling. The thermal desorption method is appropriate for use with porous polymers, particularly with Porapak N. Several investigators have used this technique (Campbell and Moore, 1979; Russell, 1975; Hughes and Horn, 1977). The advantages of thermal desorption are that it avoids manual sample pretreatment and the recovery of acrylonitrile is almost quantitative (Campbell and Moore, 1979; Russell, 1975). The method also has a higher sensitivity than the solvent desorption method, where only a fraction of the eluted acrylonitrile can be injected into the gas chromatograph. The disadvantages of the thermal desorption method include its inability to afford replicate analysis of the same sorbent tube and its tendency to cause other gas chromatography separation problems such as shortening retention time and broadening eluted peak due to the presence of adsorbed moisture on the sorbent column (Russell, 1975).

The solvents that have been used for desorption of acrylonitrile from activated carbon are acetone, methanol, carbon disulfide, and 2% acetone in carbon disulfide. The selection of the desorption sorbent is dictated by two considerations, namely, the sorbent desorption efficiency and its compatibility with gas chromatography. For example, if column separation is not adequate, methanol that has a high response on flame ionization detectors (FID) will produce a large peak shadowing the acrylonitrile peak; therefore, it is not very compatible with flame ionization detectors. Although carbon disulfide appears to be compatible with flame ionization detectors, it is a poor solvent for nitrogen/phosphorous detectors. Acetone is the solvent of choice in the latter case.

The recovery efficiencies of acrylonitrile from activated carbon with various solvents are given in Table 4-2.

Table 4-2. Recovery of Acrylonitrile from Various Solvents

| Solvent | % Recovery | Reference |
|---|---|---|
| Methanol | ca. 50% | Going et al., 1979 |
| Acetone | 73.5 ± 5.3% | Marano et al., 1978 |
| 2% acetone in $CS_2$ | 95.5 ± 7.9% | Gagnon and Posner, 1979 |
| 2% acetone in $CS_2$ | 94% | Silverstein, 1977 |
| $CS_2$ (2 ml) | 58% | Silverstein, 1977 |
| $CS_2$ (4 ml) | 75% | Silverstein, 1977 |

It should be recognized that the recovery of acrylonitrile is dependent on the nature of the activated carbon and the extent of loading. Using three different activated carbons and a variable loading of 2 μg to 200 μg acrylonitrile, Going et al. (1979) determined that the $CS_2$ desorption efficiency varied from 53% to almost 100%. It would appear from Table 4-2 that a 2% acetone in $CS_2$ is the best solvent for elution of acrylonitrile from activated carbon in terms of both recovery and GC-FID compatibility.

When the acrylonitrile is collected in liquid trapping media, the samples from high concentration sources usually do not require any pretreatment prior to the detection and quantification procedures.

### 4.1.2.2 Identification and Quantification

The methods utilized for the identification and quantification of acrylonitrile collected by sorbent or trapping techniques are shown in Table 4-1 (see 4.1.1). The methods for the analysis of acrylonitrile collected without preconcentration appear in Table 4-3. The infrared techniques are used exclusively for samples that need no preconcentration. Although a number of methods including

Table 4-3. Direct Analysis of Acrylonitrile

| Sample | Sample Introduction into Detector Unit | Analysis Technique | Confirmation Technique | Detection Limit | References |
|---|---|---|---|---|---|
| Simulated occupational air | Sample probe and pump | IR-microcomputer | None | 0.2 ppm | Jacobs and Syrjala, 1978 |
| Occupational air | Introduction of air from gas bag into evacuated cell | IR | None | 0.5 ppm | AIHA, 1970 |
| Acrylonitrile in $N_2$ | Introduction to a vacuum cell at 8 torr | IR lasers | None | 0.03 ppm | Sweger and Travis, 1979 |
| Simulated air | Pump and rotometer | Detector tube | None | Range 1-120 ppm | Kobayashi, 1956 |
| Gases generated by heating ABS plastic | Force air | Detector tube | GCMS | --- | Grote et al., 1978 |
| Occupational air | Passive diffusion | Abcor GASBADGE$^{TM}$ | None | Range 0.8- ppm | Silverstein, 1977 |
| Simulated air | Direct injection | GC-NPD | MS | 100 ppb | Marano et al., 1978 |
| Occupational air | Direct injection | GC-FID | None | 0.5 ppm | AIHA, 1970 |

GC-MS = gas chromatography-mass spectrometry
GC-NPD = gas chromatography-nitrogen/phosphorus detectors
MS = mass spectrometry
GC-FID = gas chromatography-flame ionization detectors

colorimetric, titrimetric, and polarographic were used for the analysis of acrylonitrile in the past, the GC method is most extensively used at the present time. The reason for this is the lower interference and higher sensitivity of detection obtained from GC-flame ionization detection (FID) and GC-nitrogen/phosphorus detection (NPD). The chromatographic columns found to be most appropriate for use with thermally desorbed acrylonitrile were Porapak N (Russell, 1975) and Porapak Q (Campbell and Moore, 1979). In the case of solvent desorption, the columns that were found to be suitable were Durapak OPN/ Poracil C (Going et al., 1979), SP-2100 (Grote et al., 1978) for carbon disulfide; SP-1000 (Marano et al., 1978) for acetone; and SP-1000 (Gagnon and Posner, 1979) and TCEP (Marrs et al., 1978) for methanol. The details of other chromatographic columns are given in subsection 4.7.

### 4.1.3 Conclusions

The detectors available at present often do not have sufficient sensitivity to allow the detection of acrylonitrile in ambient atmospheric samples, if these samples have been collected without preconcentration. The low level of acrylonitrile in atmospheric samples virtually mandates the use of a preconcentration device during sample collection. Of the two preconcentration methods presently available, namely, solid sorbents and trapping media, the former method is preferable to the latter. The collection of acrylonitrile by solid sorbents affords convenience in handling, shipping, and storage of the samples.

Activated carbon and porous polymer Porapak N are the two best sorbents available for the collection of acrylonitrile in air. The breakthrough capacity for Porapak N (3 to 5 liters) is lower than that for activated carbon (over 1000 liters). Consequently, Porapak N cannot be used for 24 hour sampling. Even with much lower sample volume, Porapak N will afford a detection limit comparable to that obtained from a larger volume of air collected by activated carbon, for the following reasons. The efficiency of thermal desorption for acrylonitrile from Porapak N is quantitative, while it is poor with activated carbon; therefore, activated carbon requires solvent desorption. Whereas the entire sample can be injected into the quantitative gas chromatography column in the case of thermal desorption, in the case of solvent desorption, only a fraction of the total sample can be injected.

With activated carbon, 2% acetone in carbon disulfide is the best desorption solvent, since it gives the maximum recovery. This solvent system is compatible with flame ionization detectors. Carbon disulfide, however, is unsuitable when the more sensitive nitrogen/phosphorus detectors are used. Acetone is the most suitable solvent in this case. A number of columns including SP-2100 (Gagnon and Posner, 1979) and Durapak OPN/Poracil C (Going _et al_., 1979) have been used as the quantitative column when carbon disulfide or 2% acetone in carbon disulfide was the solvent. For acetone solvent, SP-1000 was found to be a suitable quantitative column (Marano _et al_., 1978).

## 4.2 WATER

In this section, only water samples obtained from either surface water or treated drinking water will be discussed. Wastewaters will be discussed in subsection 4.3.

### 4.2.1 Sampling

Water samples have been collected almost exclusively by the grab technique (Kopfler _et al_., 1976; Wronski and Zbigniew, 1974; Going _et al_., 1979). In a few instances, multiple grab samples were composited for analysis (Going _et al_., 1979). Proposed EPA methods require that the samples be adjusted to pH 7.0 ± 0.5 and be collected in screw-cap vials with Teflon® septa without headspace. The collected samples should be stored in a refrigerator (U.S. EPA, 1979a; U.S. EPA, 1979b; U.S. EPA, 1980a). The ASTM (1980) procedure allows headspace, but recommends immediate freezing of the samples. However, a better method suggested that the samples should be collected in brown glass bottles with Teflon-lined caps and acidified at the site to a pH $\leq$ 4 (Going et al., 1979). The samples should be maintained at 0 to 4°C by ice or an ice substitute during transportation (Going et al., 1979; Kopfler et al., 1976).

In one instance (Going et al., 1979), an attempt was made to collect acrylonitrile from water by the use of solid sorbents. Four different sorbents, namely, activated carbon, Porapak N, Chromosorb 101, and Chromosorb 104, were tried. Water spiked with acrylonitrile was passed through the sorbents at a rate of 4 ml/min. The sorbents were then eluted with 25 ml methanol at a rate of 5 ml/min. The recoveries of acrylonitrile were poor; with activated carbon, the recovery was 30-35 percent and the porous polymers showed zero percent recovery.

### 4.2.2 Analysis

#### 4.2.2.1 Sample Treatment

Some water samples were analyzed without any pretreatment. Water samples containing low levels of acrylonitrile were pretreated in order to concentrate the acrylonitrile. Three available methods for concentration are purge-trap (Kopfler et al., 1976; Going et al., 1979), distillation (Going et al., 1979; Peters, 1979), and headspace (McNeil and Brader, 1981; Markelor et al., 1981) techniques.

Acrylonitrile can be purged from water at elevated temperatures by passing an inert gas through it. The acrylonitrile contained in the purged gas is then trapped in chromatographic media for subsequent analysis. The details of the purging system were described by Going et al. (1979) and Kopfler et al. (1976). The system used by Kopfler et al. (1976) appears to be preferable to the system used by Going et al. (1979), because the former allows purging of 140 ml of water compared to 10 ml water in the latter case; however, the purge-trap efficiency for acrylonitrile was not studied in detail in the Kopfler et al. (1976) system.

The purging conditions were studied in detail by Going et al. (1979), who found an almost quantitative recovery when helium was passed at a rate of 20 ml/min through water heated to 85°C for 30 minutes. The effect of different trapping systems were also studied by Going et al. (1979). It was established that, under thermal desorption conditions, both Porapak N and Chromosorb 104 gave quantitative desorption. The recovery from Tenax GC, however, was found to be poor (Going et al., 1979).

Two different distillation techniques, namely steam distillation and azeotropic distillation, have been used to concentrate acrylonitrile from the aquatic phases. In the steam distillation technique, the sample is directly distilled for an appropriate interval and the organic of interest is collected in the distillate. Peters (1980) has used this technique to concentrate acrylonitrile by a factor of 300 in about 15 minutes of steam distillation. The major disadvantage of this method and the azeotropic distillation is that relatively large sample volumes (>300 ml) are required for these methods.

In the azeotropic distillation technique, water containing acrylonitrile is distilled with methanol, and a small volume of the azeotropic distillate containing acrylonitrile in methanol is collected for further analysis. This technique serves as a simultaneous clean-up and concentration device for acrylonitrile in water samples. The description of the distillation apparatus was given by Going et al. (1979), who obtained maximum recovery under the following conditions.

A 500 ml water sample was added to the distillation flask along with 25 ml methanol and 5 ml 18N $H_2SO_4$ and the content was distilled at a rate of 1 ml/min. The first 10 ml of the distillate was collected for subsequent analysis. The percent recovery was about 90% for the combined first and second 10 ml aliquot of the distillate.

In the headspace method, a known volume of the aqueous sample is introduced into a specially designed enclosed glass apparatus and this system is thermostatically maintained at a constant temperature. After the system attains equilibrium, a known volume of the headspace vapor is withdrawn for acrylonitrile determination. This method in the past had faced problems owing to the difficulty in establishing a calibration procedure. The partition coefficient of a component between gas and liquid phase is dependent on the total ionic strength of the solution. Therefore, the same concentrations of a component present in two aqueous solutions of different ionic strengths but otherwise identical conditions will not produce the same equilibrium vapor pressure. This problem of a calibration curve has been largely obviated through the development of a standard addition method. The headspace method has been utilized by Markelor _et al._ (1981) and McNeil and Brader (1981) for the analysis of acrylonitrile in aqueous solutions.

### 4.2.2.2 Detection and Quantification

With the exception of one case in which a titrimetric method was used (Wronski and Zbigniew, 1974), the rest of the studies reviewed utilized GC separation and GC retention data for the identification of acrylonitrile from water samples. For direct aqueous injection, both Chromosorb 101 (Going _et al._, 1979) and Chromosorb 102 (Marano _et al._, 1978) were used, although Going _et al._ (1979) reported better separability with Chromosorb 101. The same column (Chromosorb 101) was used for acrylonitrile determination by the purge-trap and azeotropic distillation techniques (Going _et al._, 1979; Kopfler _et al._, 1976; _Federal Register_, 1979). Other GC columns including carbowax 1500 and Tenax GC have been used for the separation of acrylonitrile (Markelov _et al._, 1981;

Peters, 1980). An aliquot of the distillate from azeotropic distillation was injected directly into the GC column. In the purge-trap technique, the acrylonitrile from the trapping column was thermally desorbed onto the separating column. A summary of water analysis techniques is given in Table 4-4. Although nitrogen/phosphorus detectors (including the Hall detector) and flame ionization detectors were used for quantification, the former detectors (nitrogen/phosphorous) have better selectivity and sensibility than the latter detector. However, the best available method for the unambiguous identification of acrylonitrile is provided by the more expensive mass spectrometric method.

### 4.2.3 Conclusions

The two best available methods for the determination of acrylonitrile in water are the purge-trap and the azeotropic distillation techniques. Both methods gave almost quantitative recovery of acrylonitrile in water. The detection limit for acrylonitrile by the purge-trap method was lower than that for the azeotropic distillation method; however, this advantage of the purge-trap technique is somewhat offset by the experimental complexity of the method and its inability to perform replicate analysis on the same water sample.

## 4.3 WASTEWATER

### 4.3.1 Sampling

No details regarding the sampling of wastewaters are available. Grab samples may be suitable in certain cases. To monitor the discharges that are dependent on process operation stages, a 24-hour composite sample is preferable.

### 4.3.2 Analysis

#### 4.3.2.1 Pretreatment

Azeotropic distillation with methanol is a method used frequently for wastewater. This technique allows concentration of acrylonitrile and reduces the possibility of interference. In one case, solvent extractions using benzene,

Table 4-4. Analyses of Acrylonitrile in Water

| Sample | Collection Method | Analysis Technique | Confirmatory Technique | Detection Limit | Reference |
|---|---|---|---|---|---|
| Spiked water | --- | Direct injection, GC-NPD | MS | 10 ppb | Marano et al., 1978 |
| Drinking water | Grab | Purge-trap (Tenax GC), GC-MS | None | Qualitative | Kopfler et al., 1976 |
| Surface water | Grab | Titrimetric with mercaptoethanol > $Na_2SO_3$ | None | 0.5-0.05 ppm | Wronski and Zbigniew, 1974 |
| Surface water | Grab | Direct injection, GC-FID | MS | 1 ppm | Going *et al*., 1979 |
| Surface water | Grab | Purge-trap, GC-FID, > GC-Hall | MS | 0.1 ppb | Going *et al*., 1979 |
| Surface water | Grab | Azeotropic distillation, GC-Hall | MS | 0.1-1.3 ppb | Going *et al*., 1979 |
| Spiked water | Sorbent adsorption | Methanol desorption, GC-Hall | None | Showed poor desorption efficiency > method abandoned | Going *et al*., 1979 |
| Spiked water | --- | Steam distillation, GC-FID | None | NR | Peters, 1980 |
| Spiked water | --- | Headspace, GC-Hall | None | NR | Markelov et al., 1981 |

MS = Mass spectrometry
GC-NPD = gas chromatography-nitrogen/phosphorus detectors
GC-MS = gas chromatography-mass spectrometry
GC-FID = gas chromatography-flame ionization detectors
GC-Hall = gas chromatography-Hall detector
NR = Not Reported

ether, and isobutyl acetate were used (Ponomarev et al., 1974). The recovery of acrylonitrile after three extractions was not quantitative but was reproducible.

The various pretreatment methods used for acrylonitrile determination in waste water are shown in Table 4-5. Table 4-5 also lists the different detection and quantification methods and their detection limits where available. The principles of the detection methods are discussed in subsections 4.2.2.2 and 4.7.

### 4.3.3 Conclusions

Although the analysis of acrylonitrile in wastewater by the purge-trap technique has not been reported, it is a potentially appropriate method for wastewater analysis. The usefulness of the method can be enhanced by fractional purging at different temperatures (Kopfler et al., 1976). A better method for acrylonitrile analysis in wastewater is the azeotropic distillation technique. The detection and quantification can be best achieved by either the GC-FID or the GC-NPD technique. The chromatographic columns used for water analysis should be suitable for wastewater analysis.

## 4.4 SOIL AND SEDIMENT

Only one reference could be found in the literature for the analysis of acrylonitrile in these media. The following discussion in this section is based on the work of Going et al. (1979).

### 4.4.1 Sampling

Soil from the top 12 cm was removed and placed in glass bottles and stored over dry ice until analyzed. Sediment samples were collected with a dredge and the samples were kept on dry ice until analyzed.

### 4.4.2 Analysis

#### 4.4.2.1 Pretreatment

The pretreatment of the sediment samples after the removal of excess water was the same as that used for soil samples. The samples were extracted with

Table 4-5. Analysis of Acrylonitrile in Wastewaters

| Sample | Pretreatment | Analysis treatment | Detection Limit | Reference |
|---|---|---|---|---|
| --- | --- | Polarographic | 10 ppm | Sevest'yanova *et al*., 1966 |
| --- | Solvent extraction | Polarographic | 3-5 ppm | Ponomarev *et al*., 1974 |
| --- | Azeotropic distillation | Polarographic | <0.1 ppm | Daues and Hamner, 1957 |
| --- | Azeotropic distillation | Titrimetric | <2 ppm | Stefanescu and Ursu, 1973 |
| --- | None | Colorimetric | <0.5 ppm | Lawniczak, 1977 |
| --- | Azeotropic distillation | Titrimetric | --- | Covic-Horvat *et al*., 1970 |
| From polymerization process | None | GCFID | 0.1 ppm | Deur-Siftar and Svob, 1976 |

GC-FID = gas chromatography-flame ionization detectors water, using ultrasonic agitation, and the mixtures were centrifuged. The supernatants were withdrawn and filtered, and the aqueous extracts were directly used for analysis. A few samples were concentrated by the purge-trap technique already discussed in subsection 4.2.2.1. It should be mentioned that the overall recovery of acrylonitrile from the soil and sediment samples either by direct extraction or by the subsequent purge-trap technique was not determined.

#### 4.4.2.2 Detection and Quantification

Gas chromatography with a Chromosorb 101 column and a Hall detector was used for detection and quantification. The detection limit for direct aqueous injection ranged from 50 to 400 μg/kg. For the purge-trap technique, the detection limit was *ca*. 0.5 μg/kg.

### 4.4.3 Conclusions

The desorption efficiency of acrylonitrile from soil and sediment samples by ultrasonic agitation has yet to be determined. Although azeotropic distillation was not used, both this method and the purge-trap technique should be suitable for the determination of low levels of acrylonitrile in these samples.

## 4.5 RESIDUE IN POLYMERS AND THE EXTENT OF MONOMER MIGRATION IN FOOD-SIMULATING SOLVENTS

Several polymers of acrylonitrile are presently used in the United States as food packaging materials. Until recently, some containers for carbonated beverages had nitrile barrier resins as components; however, the use of nitrile barrier resins in beverage containers in the United States is presently banned by FDA. The ABS resins (terpolymer of acrylonitrile, butadiene, and styrene), however, are currently used for such food packages as margarine tubs, fruit juice containers, and vegetable oil bottles. In this section, the analysis of acrylonitrile residue in both polymers used for food packaging and their migration to food-simulating solvents kept in contact with the polymer will be discussed.

### 4.5.1 Analysis

### 4.5.1.1 Pretreatment

### 4.5.1.1.1 Pretreatment for Polymers

Three methods are available for the pretreatment of polymers prior to quantification. In one method, the sample is dissolved in a suitable solvent and an aliquot of this solution is used for analysis. The solvents used for different polymers are shown in Table 4-6. The disadvantage of direct injection of the solution is that it accelerates the deterioration of the separating column when GC is used for identification. The polymer build-up in the gas chromatograph injection port can be prevented by addition of water or methanol to precipitate the polymer and the supernatant can be injected into the gas chromatograph. The detection limit of this method, however, is not satisfactory and was determined to be about 10 ppm (Steichen, 1976).

To avoid column contamination, reduce the interference arising from large amounts of solvent, and increase the sensitivity of detection. The second method, known as the head-space analysis, is presently used for the determination of residual monomer in polymers.

Two approaches to the head-space analysis have been used: solid and solution. The solid approach involves the equilibration of a solid polymer sample in the sealed tube at a constant elevated temperature. The advantage of the solid approach is that it has tenfold more sensitivity than the solution approach (Steichen, 1976). The disadvantages of this approach are (a) equilibration with the head-space may take a long time, and (b) since polymer standards of known monomer content are not readily available, the head-space monomer concentration must be related to the original concentration in the polymer either by assuming 100% diffusion of the monomer into the head-space or through

Table 4-6. Analysis of Acrylonitrile Residue in Polymers and Food-Simulating Solvents

| Sample | Pretreatment | Analysis Technique | Confirmation Technique | Detection Limit | Reference |
|---|---|---|---|---|---|
| Plastic | Pyrolysis and solvent trapping | Polarographic | None | 100 ppm | Uhde and Koehler, 1967 |
| Plastic | Dissolved in benzene | Titrimetric | None | <6 ppm | Roy, 1977 |
| Residue in food-simulating solvent | Direct | GC-FID | None | --- | Markelov and Semenenko, 1976 |
| Polymer | (a) Pyrolysis GC<br>(b) Dissolution in dimethyl formamide<br>(c) Dissolved in ether | GC-FID | None | 10-100 ppm | Reichle and Tengler, 1968 |
| Residue in food-simulating solvent | Head-space equilibration | GC-FID | None | --- | Chudy and Crosby, 1977 |
| Residue in food-simulating solvent | Azeotropic distillation | GC-NPD | MS | <0.01 ppm | McNeal et al., 1979 |
| Residue in food-simulating solvent | Direct | GC-NPD | None | 0.04 ppm | Brown et al., 1978 |
| Polymer | Dissolved in N,N-dimethylacetamide | GC-NPD | None | 0.5 ppm | Brown et al., 1978 |
| Residue in food-simulating solvent | Azeotropic distillation | GC-NPD | None | 50 ppb | Hartshorn, 1975 |
| ABS resin | Dissolved in dimethyl formamide or dimethyl sulfoxide and head-space equilibration | GC-FID and GC-NPD | None | 0.3 ppm | DiPasquale et al., 1978 |
| Residue in food-simulating solvent | Head-space equilibration | GC-NPD | None | 30 ppb | DiPasquale et al., 1978 |

Table 4-6. Analysis of Acrylonitrile Residue in Polymers and Food-Simulating Solvents (cont.)

| Sample | Pretreatment | Analysis Technique | Confirmation Technique | Detection Limit | Reference |
|---|---|---|---|---|---|
| Polymer | Dissolved in o-dichlorobenzene and head-space equilibration | GC-FID | None | 0.5 ppm | Steichen, 1976 |
| Plastic | Dissolved in propylene carbonate and head-space equilibration | GC-NPD | None | 0.1 ppm | Gawell, 1979 |
| Residue in beverages | Head-space equilibration | GC-NPD | None | 5 ppb | Gawell, 1979 |

GC-FID = gas chromatography-ionization detectors
GC-NPD = gas chromatography-nitrogen/phosphorus detectors

determination of equilibrium concentration using Henry's law and the appropriate partition coefficient.

The solution head-space approach has been used with a much wider range of samples (Steichen, 1976; Gawell, 1979; DiPasquale et al., 1978). In this case, the polymer has been dissolved in a suitable solvent and allowed to equilibrate in a sealed vial at a constant temperature (60 to 90°C). Sometimes water has been added to the solvent to enhance the equilibrium monomer concentration (Steichen, 1976). The advantages of this approach are: (a) head-space equilibration is rapid, (b) calibration procedure is simplified, and (c) the head-space gas/solution partitioning of the constituents is not appreciably affected by changes in the solvent phase.

The third method, which is rarely used at the present time, consists of passing nitrogen gas through the heated polymers and trapping the volatile components in dimethyl formamide (Uhde and Koehler, 1967). The dimethyl formamide solution is subsequently analyzed for acrylonitrile. A variation of this method called the pyrolysis GC technique has also been used (Reichle and Tengler, 1968).

#### 4.5.1.1.2 Pretreatment of Food-Simulating Solvent Containing Monomer Residue

The pretreatment of food-simulating solvents for the determination of monomer content as a result of migration from the polymer has been done in three ways. The first method that has been used is the direct analysis of the solvent left in contact with the polymer for a predetermined time and temperature (Markelov and Semenenko, 1976; Brown et al., 1978). The disadvantage of this method was that it had a poor sensitivity and the large amount of solvent interfered with the small amount of acrylonitrile during the detection and quantification stage (Brown et al., 1978; Hartshorn, 1975).

To avoid the solvent interference and to enhance the sensitivity of detection, either the second or the third method, namely azeotropic distillation or head-space analysis, is presently employed.

In the azeotropic distillation technique, the solvent is distilled with methanol and the azeotrope containing methanol and acrylonitrile is collected for further analysis. When water or 8% ethanol was used as the food-simulating solvent, the distillation was done by adding methanol directly to the solvent (McNeal et al., 1979). When 3% acetic acid was used as the food-simulating solvent, the solution was neutralized with sodium hydroxide before distillation with methanol (McNeal et al., 1979). When heptane was used as the food-simulating solvent, it was extracted with water and the water extract was distilled with methanol (McNeal et al., 1979). The average recovery of acrylonitrile from the azeotropic distillation of all the extracts varied between 29.7 ± 2.6% and 32.6 ± 2.8% (McNeal et al., 1979).

The third method employs the principle of the head-space equilibration technique. The solvent was introduced in a sealed vial and allowed to equilibrate at a predetermined temperature for a certain length of time (Chudy and Crosby, 1977; DiPasquale et al., 1978; Gawell, 1979).

#### 4.5.1.2 Detection and Quantification

Although a polarographic (Uhde and Koehler, 1967) and a titrimetric method (Roy, 1977) have been used for the determination of monomer in polymers, these methods lacked adequate sensitivity for the determination of low levels of acrylonitrile. The method used almost exclusively at the present time is gas chromatography, either with flame ionization detectors or with nitrogen/phosphorus detectors. The sensitivity and selectivity of NPD makes it a preferable method over FID.

### 4.5.2 Conclusions

The best available method for the determination of monomer residue in polymers seems to be the solution head-space and GC analysis. For the determination of the extent of monomer migration in food-simulating solvents, both azeotropic distillation and solution head-space equilibration with subsequent GC analysis are the preferred methods. The preferred stationary phases that have been used for non-aqueous injections are Carbowax-20M and Carbowax-1500 (Chudy and Crosby, 1977; DiPasquale _et al._, 1978; Steichen, 1976; Gawell, 1979). For aqueous injections, gas-solid chromatography with porous polymer packings such as Chromosorb-102 (DiPasquale _et al._, 1978), Chromosorb-101 and -108 (McNeal _et al._, 1979) and Porapak-QS and -S (Brown _et al._, 1978) have been used.

## 4.6 OTHER MEDIA

Acrylonitrile levels have been determined in many other media such as tobacco, production streams, and foods and grains consumed by humans. Since acrylonitrile-containing pesticides have been voluntarily withdrawn from the market and the other media have no direct bearing on general population exposure, acrylonitrile analysis in these media will not be discussed.

## 4.7 GENERAL METHODS FOR THE ANALYSIS OF ACRYLONITRILE

This section presents the general methods available for detection and quantification of acrylonitrile without regard to the medium in which it is present. Many of the methods have been applied for analysis of acrylonitrile present in more than one medium. This approach has been adopted so that replication of discussion of the same method from medium to medium is avoided.

The identification and quantification methods can be divided into two categories: one based upon the chemical reactivities of the functional groups in acrylonitrile and the other based upon instrumental techniques. The chemical techniques that have proved useful are based on (a) hydrolysis of the nitrile group and (b) additions to the double bond. In the hydrolysis method, acrylonitrile is hydrolyzed to ammonia and acrylate ion by a strong base. The resulting ammonia can be determined colorimetrically by the Nessler's method (Aarato and Bittera, 1972; AIHA, 1970) or by NaOCl and Na-salicylate in the presence of Na-nitroferricyanide (Rogaczewska, 1976). Alternatively, the liberated ammonia can be determined by titrimetric method (Gunther and Blinn, 1955).

Colorimetric, titrimetric, and thin layer chromatographic procedures have been developed based upon the addition reaction of acrylonitrile. In one colorimetric procedure, acrylonitrile is brominated with (Krynska, 1960; Russkikh, 1971; Lawniczak, 1977) or without (Russkikh, 1973; Nazarova and Nakrap, 1978) u.v. radiation. The excess bromine is neutralized and the cyanogen bromide that is formed is allowed to react with a benzidine-pyridine solution to form a colored complex (Nazarova and Nakrap, 1978). In another method, the acrylonitrile complex that is formed with pyridine in the presence of a basic hypochlorite solution at 60-65°C is measured at 411 nm (Hall and Stevens, 1977).

The titrimetric procedures have been used following the reaction of acrylonitrile with excess $Na_2SO_3$ (Burkart et al., 1961; Terent'ev and Obtemperanskaya, 1956; Taubinger, 1969), thioglycolic acid (Stefonescu and Ursu, 1973; Covic-Horvat et al., 1970), $NaHSO_3$ (Kostin and Vidanova, 1957), dodecanethiol (Roy, 1977), or lauryl mercaptan (Berck, 1975). The excess reagent is then back-titrated with the appropriate titrant in the presence of an indicator or by the potentiometric method.

A method based on the interaction of acrylonitrile with alkaline $KMnO_4$ solution, which produces a change in the permanganate color, has been used to determine the concentration of acrylonitrile. In this method, the concentration was determined by comparison of the color from a calibration curve (Gisclard et al., 1958).

In the thin layer chromatography (TLC) procedure, the mercuric acetate adduct of acrylonitrile was separated by TLC on silica gel. The solvent system used was described by Braun and Vorendohre (1963). In a recent method (Plieninger and Sharma, 1978), the indole adduct of acrylonitrile was separated by TLC procedure.

The instrumental techniques for identification and quantification have used gas chromatography, polarography, infrared and u.v. spectroscopy, and mass spectrometry. Of these methods, mass spectrometric is the most unambiguous method for the identification of acrylonitrile.

The vast majority of GC procedures have relied on flame ionization detection (FID). Electron capture detection resulted in one-fifth the sensitivity of FID (Barrett, 1974, cited in Going et al., 1979). The use of nitrogen/phosphorus detectors (NPD) has resulted in a dramatic increase in the sensitivity of detection. The insensitivity of this detector toward compounds that do not contain nitrogen and phosphorus eliminates interferences from many compounds (DiPasquale et al., 1978). Neither carbon disulfide nor N-containing solvents are suitable for NPD.

The selection of column packing materials for the separation of acrylonitrile from interferences depends on the source of the sample. Numerous packing materials have been used in the past. Based on their chemical characteristics, the stationary liquid or solid phases that have been used for the analysis of acrylonitrile can be summarized as follows:

> Polyglycol: (a) Carbowax-1500 (Babina, 1979; DiPasquale et al., 1978; Steichen, 1976; Gawell, 1979) (b) Carbowax-1540 (Chudy and Crosby, 1977) (c) Carbowax-400 (Lysyj, 1960; DiLorenzo and Russo, 1969) (d) Carbowax-20M (Chudy and Crosby, 1977; DiPasquale et al., 1978; Gawell, 1979) (e) SP-1000 (Marano et al., 1978; Gagnon and Posner, 1979) and (f) Di-glycerol (Ustinovskaya et al., 1977)

Hydrocarbon: (a) Apiezon (Babina, 1979) (b) Tween-80 (Chopra *et al.*, 1978)

Esters and Polyesters: (a) Polyethylene glycol adipate (Panova *et al.*, 1969; Kleshcheva *et al.*, 1971; Markelov and Semenenko, 1976) (b) Neopentyl glycol succinate (Kleshcheva *et al.*, 1971; Korzhova *et al.*, 1974) (c) Triethylene glycol butyrate (Pokrovskaya and Frolova, 1969) (d) Polyethylene glycol succinate (Lysyj, 1960) (e) Dioctyl phthalate (Nestler and Berger, 1965)

beta-beta'-oxydipoprionitrile (Pokrovskaya and Frolova, 1969; Reichle and Tengler, 1968; DiLorenzo and Russo, 1969)

Silica gel ASK (Ivanenko and Lukashevaskaya, 1976)

Porous Polymers: (a) Porapak Q (Balak *et al.*, 1977; Campbell and Moore, 1979) (b) Porapak QS (Brown *et al.*, 1978) (c) Porapak N (Russell, 1975; Tanaka *et al.*, 1975) (d) Porapak S (Brown *et al.*, 1978) (e) Chromosorb 101 (McNeal *et al.*, 1979; Brown *et al.*, 1978) (f) Chromosorb 102 (Marano *et al.*, 1978; DiPasquale *et al.*, 1978) (g) Chromosorb 104 (Going *et al.*, 1979) (h) Chromosorb 108 (McNeal *et al.*, 1979)

Methylsilicone: (a) SE 30 (Berck, 1965) (b) DC-200 (Beaumont and Garrido, 1979)

Aminoalcohol: THEED (Hughes and Horn, 1977)

Tetracyanoethyl: Pentaerytritol (Ustinovskaya *et al.*, 1977; Deur-Siftar and Svob, 1976; Marrs *et al.*, 1978)

The polarographic method was used most extensively in the past for the determination of acrylonitrile. Tetramethylammonium iodide (Sevest'yanova and Tomilov, 1963; Gorokhovskaya and Geller, 1962; Uhoe and Koehler, 1967; Chao and Ch'en, 1966; Daues and Hamner, 1957; Lezovic and Singliar, 1977; Mekhtiev *et al.*, 1968; Klyaev *et al.*, 1966; Rogaczewska, 1964), tetramethylammonium hydroxide (Berck, 1962; Sevast'yanova *et al.*, 1966), and LiCl (Sevest'yanova and Tomilov, 1963; Bogaczek and Jaworski, 1970) have been used as the supporting electrolyte for the dropping mercury electrode. Both standard calomel and silver were used as the reference electrode. A continuous polarographic method has been used for the determination of acrylonitrile in industrial streams (Bogaczek and Jaworski, 1970).

Spectroscopic methods using both infrared and ultraviolet techniques have been used for the detection and quantification of acrylonitrile. The sensitivity of acrylonitrile determination by the earlier IR methods was rather poor (Scheddel, 1958; Karaenev et al., 1974). In recent years, however, the use of multiple reflection, which, in essence, has the effect of increasing the cell path-length, has increased the sensitivity significantly (Kurapov et al., 1977; AIHA, 1970; Beaumont and Garrido, 1979). The same principle has been applied for the continuous monitoring of acrylonitrile at 10.5 nm with a portable infrared analyzer (Jacobs and Syrjala, 1978).

An infrared laser technique, called laser stark spectroscopy, that applies electric fields to perturb the molecular rotational energy levels has been used to enhance and modulate the absorption of acrylonitrile. Using the P(28) line from a $CO_2$ laser and a 40 cm IR cell, the method was shown to detect 0.03 ppm of acrylonitrile in air (Sweger and Travis, 1979).

Acrylonitrile in solution has been determined by u.v. absorption at 195 nm (Petrova et al., 1978) and at 210 nm (Brieger et al., 1952).

Mass spectrometry alone is rarely employed for the quantification of acrylonitrile. It is usually used as a confirmatory technique. In one study, however, it was used for monitoring acrylonitrile concentration in process streams (Thomson, 1974). In combination with GC, mass spectrometry was used for confirmatory identification (Grote et al., 1978; Marano et al., 1978; Tanaka et al., 1975; Going et al., 1979; McNeal et al., 1979). The use of multiple ion monitoring mode increases the detection limit five-fold over full mass scan mode (McNeal et al., 1979).

Finally, detector tubes for area monitoring (Kobayashi, 1956) and gas badges for personnel monitoring (Silverstein, 1977) of acrylonitrile have been proposed.

# 5. SOURCES IN THE ENVIRONMENT

## 5.1 PRODUCTION PROCESSES

Acrylonitrile can be produced by the following methods:

(a) Oxidation of propylene in the presence of ammonia (ammoxidation of propylene) using either a bismuth phosphomolybdate or uranium-base catalyst;

(b) Addition of hydrogen cyanide to acetylene using a cuprous chloride catalyst;

(c) Catalytic reaction of propylene with nitrous oxide;

(d) Reaction of ethylene oxide with hydrogen cyanide, followed by catalytic dehydrogenation of ethylene cyanohydrin; and

(e) Ammoxidation of propane.

Processes (a) through (d) have been used for the commercial production of acrylonitrile, and process (e) has been studied on a pilot scale (Hughes and Horn, 1977). Since 1971, however, process (a) (the ammoxidation of propylene) is the only process that has been used commercially in the United States. The process is patented by the Standard Oil Company of Ohio (SOHIO) and is known as the SOHIO process.

## 5.2 ACRYLONITRILE PRODUCERS

The producers of acrylonitrile monomer in the United States are given in Table 5-1, while distribution for 1980 and growth projections through 1984 figures are given in Table 5-2.

Table 5-1. Producers of Acrylonitrile in the United States (Crume, 1982)

| Producer | Capacity, Mg x $10^3$ |
|---|---|
| American Cyanimid<br>Westwego, LA | 123 |
| DuPont<br>Beaumont, TX | 159 |
| DuPont<br>Memphis, TN[a] | 136 |
| Monsanto<br>Alvin, TX | 113 |
| Monsanto<br>Texas City, TX | 191 |
| Vistron<br>Lima, OH | 136 |
| Vistron<br>Victoria, TX | 270 |
| TOTAL | 1,128 |

[a] Dupont has announced plans to close this plant (C and EN Oct. 11, 1982)

Table 5-2. Distribution of Acrylonitrile in 1980 and Projected Growth Through 1984 (*Chemical Marketing Reporter*, 1980)

| Distribution | Acrylonitrile (Mg x $10^3$)[a] | Projected Annual Growth |
|---|---|---|
| Consumption | 664 | 4% |
| Imports | Negligible | -- |
| Exports | 198 | Decline |

[a] Production at Vistron, Victoria, Texas, is not included

## 5.3 ACRYLONITRILE USES

Acrylonitrile is used primarily as a raw material in the synthesis of acrylic and modacrylic fibers, ABS and SAN resins, nitrile rubbers, adiponitrile, acrylamide, and barrier resins. Other miscellaneous uses include the production of fatty amines and their derivatives, cyanoethylation of various alcohols and amines, fumigant formulations, as an absorbent, and as an anti-stall

Table 5-3. Primary Uses for Compounds Synthesized from Arylonitrile-Containing Compounds (Suta, 1979)

| Compound | Uses |
|---|---|
| Acrylic and Modacrylic Fibers | More than 60% of these fibers is used in apparel. Carpeting is the second largest use. Home furnishing uses include blankets, draperies, and upholstery. Industrial uses include sandbags, filter cloths, tents, and tarpaulins. The fibers are also used in synthetic hair wigs. |
| ABS Resin | Its major markets are pipes and pipe fittings, and automotive components. Other important markets are large appliances, housing for business machines and telephones, recreational vehicle components, toys, sporting goods, sheeting material for luggage, and food containers. |
| SAN Resin | Its primary uses are for drinking tumblers and other houseware items, for automobile instrument panels, instrument lenses, and food containers. |
| Nitrile Elastomers | Its major uses are in rubber hose, seals, gaskets, latex, adhesives, polyvinyl chloride blending, paper coatings, and pigment binders. |
| Adiponitrile | It is hydrogenated to hexamethylenediamine, which is used to produce nylon. |
| Acrylamide | Its largest use is in the production of polyacrylamides for waste and water treatment flocculants. Other acrylamide products are used to aid sewage dewatering, and for papermaking strengtheners and retention aids. |
| Nitrile Barrier Resins | They are used in the manufacture of non-beverage containers for glue, nail polish, correction fluid, air freshener, contact lenses, tooth brushes, and combs (Miller and Villaume, 1978). |

automotive additive (Miller and Villaume, 1978). A flow diagram summarizing direct and indirect uses of acrylonitrile is given in Figure 5-1. The primary uses of the compounds that are synthesized from acrylonitrile-containing compounds are presented in Table 5-3.

## 5.4 CONSUMPTION OF ACRYLONITRILE BY PRODUCT

A breakdown of acrylonitrile consumption and projected growth of products using acrylonitrile in their manufacture is given in Table 5-4.

Table 5-4. Acrylonitrile Consumption and Project Growth of Products Using Acrylonitrile

| Product | 1977 Consumption (Mg x $10^3$)[a] | Projected Annual Growth Through 1982(%)[a] | 1980 Consumption (Mg x $10^3$)[b] | Projected Annual Growth Through 1984[b] |
|---|---|---|---|---|
| Acrylic and modacrylic fibers | 331 | 4.5-5.5 | 345 | None |
| ABS and SAN resins | 142[c] | 7.5-9.5 | 172 | Some Growth |
| Nitrile elastomers | 24 | 2.0-3.0 | -- | -- |
| Adiponitrile | 73 | 10.5-12.5 | -- | -- |
| Acrylamide | 24 | 8.0-10.0 | -- | -- |
| Nitrile barrier resins | 9 | 12.0 | -- | -- |
| Other | 33 | 4.0-6.0 | 147 | Decline |

[a] Suta, 1979

[b] CMR, 1980

[c] 126 x $10^3$ Mg for ABS and 16 x $10^3$ Mg for SAN resins

The U.S. manufacturing plants that use acrylonitrile in production (except for the plant producing adiponitrile) are listed in Tables 5-5 through 5-8. The only U.S. producer of adiponitrile from acrylonitrile is Monsanto. Their facility at Decatur, Alabama, produced 67 x $10^3$ Mg of adiponitrile in 1977 (SRI, 1978).

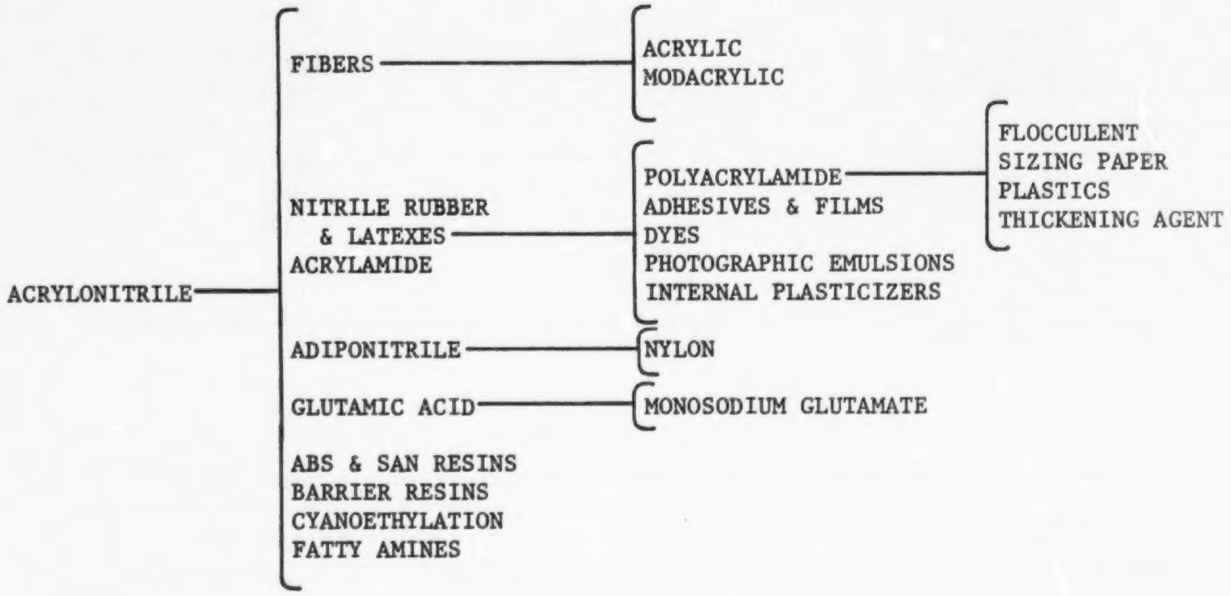

ACRYLONITRILE
FIBERS
ACRYLIC
MODACRYLIC
NITRILE RUBBER & LATEXES
ACRYLAMIDE
POLYACRYLAMIDE
ADHESIVES & FILMS
DYES
PHOTOGRAPHIC EMULSIONS
INTERNAL PLASTICIZERS
FLOCCULENT
SIZING PAPER
PLASTICS
THICKENING AGENT
ADIPONITRILE
NYLON
GLUTAMIC ACID
MONOSODIUM GLUTAMATE
ABS & SAN RESINS
BARRIER RESINS
CYANOETHYLATION
FATTY AMINES

**Figure 5-1.** Flow Diagram for Acrylonitrile Usage (NIOSH, 1977c)

Table 5-5. Producers of SAN and ABS Resins (Crume, 1982)

| Producer | Capacity (Mg x $10^3$) |
|---|---|
| ABTEC (Mobay) | |
| Louisville, KY | 31.8 |
| Borg-Warner | |
| Ottawa, IL | 105 |
| Washington, WV | 136 |
| Port Bienville, MS | NA |
| Dow | |
| Allyns Point, CT | 29.5 |
| Midland, MI | 65.9 |
| Pevley, MO | 29.5 |
| Torrance, CA | 34.1 |
| Irontown, OH | 29.5 |
| Monsanto | |
| Addyston, OH | 159 |
| Muscatine, IA | 56.8 |
| Springfield, MA | 13.6 |
| USS Chemical | |
| Scotts Bluff, LA | 90.9 |

NA = Not Available

Table 5-6. Producers of Acrylic and Modacrylic Fibers (Crume, 1982)

| Producer | Capacity (Mg x $10^3$) |
|---|---|
| American Cyanamid | |
| Milton, FL | 59.1 |
| Badische | |
| Williamsburg, VA | 36.8 |
| DuPont | |
| Camden, SC | 77.3 |
| Waynesboro, VA | 61.4 |
| Tennessee Eastman | |
| Kingsport, TN | 16.4 |
| Monsanto | |
| Decatur, AL | 145 |

Table 5-7. Producers of Nitrile Rubbers and Elastomers (Crume, 1982)

| Producer | Capacity (Mg x $10^3$) |
|---|---|
| Copolymer Rubber | |
| Baton Rouge, LA | 6.2 |
| B.F. Goodrich | |
| Akron, OH | 14.1 |
| Louisville, KY | 28.6 |
| Goodyear | |
| Akron, OH | 7.5 |
| Houston, TX | 18.2 |
| Uniroyal | |
| Painesville, OH | 16.4 |
| Reichold | |
| Cheswold, DE | 10.4 |

Table 5-8. Producers of Acrylamide (SRI, 1978)

| Producers | Capacity (Mg x $10^3$) |
|---|---|
| American Cyanamid | |
| Linden, NJ | 36[a] |
| Westwego, LA | |
| Dow | |
| Midland, MI | 23 |
| Nalco | |
| Garysville, LA | 4.5 |

[a] Combined total for both plants

## 5.5 SOURCES OF EMISSIONS

Acrylonitrile emissions occur during: (1) monomer and polymer production; (2) transportation; and (3) end product usage. These emission sources are discussed below.

### 5.5.1 Monomer and Polymer Production

Acrylonitrile emission estimates for monomer, ABS-SAN resin, acrylic fiber, nitrile elastomer, and adiponitrile production are given in Tables 5-9 through 5-13, respectively. (Other acrylonitrile emission sources are relatively minor and are not included). These estimates are based on information provided by both industry and EPA, and are included in a report prepared by Suta (1982). The estimates assume full capacity; however, in recent years actual production levels have been somewhat below full capacity. Actual production levels were not used in the estimates because production levels vary considerably from year to year. Also, some plants consider their production levels proprietary information. The estimates of public exposure potential presented elsewhere in this report are based on the emission estimates contained in the Suta (1982) report.

EPA has independently calculated the emission estimates contained in the Suta (1982) report (Crume, 1982). The current EPA estimates agree closely with the Suta estimates for most acrylonitrile production plants. The current EPA estimate for total acrylonitrile emissions from the entire industry differs from the Suta total by less than +2%.

### 5.5.2. Emissions During Transportation

Estimates concerning the importance of potential acrylonitrile spills during transportation-related accidents are presented in Table 5-14. These estimates were originally developed to determine the relative costs of different modes of transportation and were not intended to indicate the magnitude of potential spills for the entire acrylonitrile industry. The estimates do suggest

Table 5-9. Estimated Atmospheric Emissions of Acrylonitrile from Monomer Production Facilities (Suta, 1982)

| Producer | Acrylonitrile Emissions (Mg/year) |
|---|---|
| American Cyanamid | |
| Westwego, LA | 94.7 |
| DuPont | |
| Beaumont, TX | 78.8 |
| Memphis, TN | 216 |
| Monsanto | |
| Alvin, TX | 95.4 |
| Texas City, TX | 162 |
| Vistron | |
| Lima, OH | 132 |
| Victoria, TX | 23.3 |
| TOTAL | 802 |

Table 5-10. Estimated Acrylonitrile Emission Rates from ABS-SAN Resin Production (Suta, 1982)

| Producer | Acrylonitrile Emissions (Mg/year) |
|---|---|
| ABTEC (Mobay) | |
| Louisville, KY | 4.5 |
| Borg Warner | |
| Ottawa, IL | 96.6 |
| Washington, WV | 657 |
| Port Bienville, MS | 1.3 |
| Dow | |
| Allyns Point, CT | 7.9 |
| Midland, MI | 17.3 |
| Pevley, MO | 1.1 |
| Torrance, CA | 9.1 |
| Irontown, OH | 6.2 |
| Monsanto | |
| Addyston, OH | 89.6 |
| Muscatine, IA | 365 |
| Springfield, MA | 10.0 |
| USS Chemical | |
| Scotts Bluff, LA | 158 |
| TOTAL | 1,424 |

Table 5-11. Estimated Acrylonitrile Emission Rates from Acrylic Fiber Production (Suta, 1982)

| Producer | Acrylonitrile Emissions (Mg/year) |
|---|---|
| American Cyanamid | |
| Milton, FL | 142 |
| Badische | |
| Williamsburg, VA | 357 |
| DuPont | |
| Camden, SC | 352 |
| Waynesboro, VA | 309 |
| Tennessee Eastman | |
| Kingsport, TN | 25.0 |
| Monsanto | |
| Decatur, AL | 91.0 |
| TOTAL | 1,276 |

Table 5-12. Estimated Acrylonitrile Emission Rates from Nitrile Elastomer Production (Suta, 1982)

| Producer | Acrylonitrile Emissions (Mg/year) |
|---|---|
| Copolymer Rubber | |
| Baton Rouge, LA | 4.3 |
| B.F. Goodrich | |
| Akron, OH | 112 |
| Louisville, KY | 63.4 |
| Goodyear | |
| Akron, OH | 55.2 |
| Houston, TX | 0.2 |
| Uniroyal | |
| Painesville, OH | 40.0 |
| Reichold Cheswold, DE | 8.4 |
| TOTAL | 295 |

Table 5-13. Estimated Acrylonitrile Emission Rate from Adiponitrile Production (Suta, 1982)

| Producer | Acrylonitrile Emissions (Mg/year) |
|---|---|
| Monsanto | |
| Decatur, AL | 59.0 |

the magnitude of potential spills from several modes of transportation, but under highly idealized circumstances. (See, for example, the footnotes to Table 5-14).

Some data on actual acrylonitrile spills have been reported to the Oil and Hazardous Materials Spill Information Retrieval System (OHM-SIRS) of the EPA. From August 1970 to July 1975, 12 acrylonitrile spills were reported to OHM-SIRS, 10 of which occurred during transport. Of these 10 spills, 7 occurred from tank cars, 2 from barges, and 1 from a tank truck. However, EPA cautions that only 10 to 20% of all spills are ever reported (Miller and Villaume, 1978). The Intergovernmental Maritime Consultative Organization estimated that 41 tons of acrylonitrile were discharged into the sea from transport and handling in 1970 (NAS, 1975, cited in Miller and Villaume, 1978).

### 5.5.3 Emissions from End-Product Usage

Another source of environmental contamination is from residual monomer release during end-product usage. Monomer residues occurring in end-products are given in Table 5-15. The level of acrylonitrile in fibers is so low that handling of the fibers is not a likely source of acrylonitrile exposure. This conclusion is supported experimentally by Finkel et al. (1979). Even if the product were heated, this would not result in a significant release of acrylonitrile (Federal Register, 1978a). Acrylonitrile may possibly be leached from fabrics during laundering; however, research has not been conducted in this area.

Research by A.T. Kearney, Inc. (Kearney, 1978) indicates that in non-food contact, ABS/SAN containers do not release any acrylonitrile under normal use; in contact with foods, however, these ABS/SAN containers may release acrylonitrile into the foods. Brown et al. (1978) determined that SAN bottles (7 ppm residual monomer content) in contact with 3% acetic acid at 49 °C for 1 month releases 0.013 ppm of acrylonitrile to the acetic acid. ABS resin (24 ppm residual

Table 5-14. Hazards of Acrylonitrile Transportation[a] (A.D. Little, Inc., 1974 as cited in Miller and Villaume, 1978)

| Hazard Parameter | Barge | Truck | Rail |
|---|---|---|---|
| Spill Pool Radius (meter) | 61.0 | 17.1 | 31.7 |
| Hazard Radius (meter) | 122 | 38.4 | 68.3 |
| Hazard Area ($m^2$) | 46,700[b]<br>5,460[d] | 4,450[c] | 13,400[c] |
| Relative Exposure (%) Urban/Rural | 8/92 | 23/77 | 27/73 |
| Expected Number of Annual Spills | 0.0117 | 0.063 | 0.17 |
| Probability of Ignition Following Spill | 0.30 | 0.25 | 0.40 |
| Expected Annual Number of People Exposed[e] Urban/Rural | 0.008/0.004 | 0.010/0.002 | 0.16/0.016 |
| Expected Annual Property Damage ($)[e] Urban/Rural | 129/55 | 160/20 | 2423/252 |
| Recurrence Interval[f] (Years) | 85.5 | 15.8 | 5.8 |

[a] Calculations are based upon the assumption that each mode of transportation handles 100 percent of the quantity shipped, and that a total of 73,000 Mg per year of acrylonitrile are shipped between two points.

[b] Area affected by spills into water which ignite. Assumes entire spill quantity contributes to burning pool.

[c] Area affected by spills on land which ignite. If no ignition occurs the exposed land area is equivalent to the pool spill area ($\pi R^2$ spill).

[d] For spills into water which do not ignite. The water toxicity hazard distance (meters) measured downstream from spill location for a 152-meter-wide, 3.05-meter-deep river flowing at 0.70 meters per second. Assumes vertical dispersion rate at 0.30 meters per minute until uniform mixing is achieved throughout the entire depth of the river. Thereafter, plug flow is assumed with no synergistic or antagonistic reaction between the pollutant and the receiving body of water. For this situation the entire spill quantity contributes to water.

[e] Expected number of people exposed annually and property damage is based upon ignition of the flammable pool for both land- and water-based spills.

[f] Average number of years between accidents.

Table 5-15. Monomer Residue in End-Products of Acrylonitrile

| Product Name | Usage | Monomer Residue (ppm) | Reference |
|---|---|---|---|
| Acrylic and Modacrylic Fiber | Fabric | <1 | Miller and Villaume, 1978 |
| Hycar | Rubber | 0-100 | Miller and Villaume, 1978 |
| Kralastic and Paracril | Resin | 50 | Miller and Villaume, 1978 |
| UCAR-380 | Latex | 250 | Miller and Villaume, 1978 |
| UCAR-4358 | Latex | 750 | Miller and Villaume, 1978 |
| Acrylamide Monomer | See Figure 5-1 | 50-100 | Miller and Villaume, 1978 |
| Polyacrylamide | See Figure 5-1 | 1 | Kearney, 1978 |
| ABS Resin | Packaging | 24 | Brown *et al.*, 1978 |
| SAN Resin | Containers | 3-7 | McNeal *et al.*, 1979 |
| SAN Resin | Containers | 2-5 | Gawell, 1979 |

monomer content) under the same conditions released 0.283 ppm of acrylonitrile (Brown et al., 1978).

Acrylonitrile may be present as an impurity in products made from acrylamide (Miller and Villaume, 1978). However, the extent to which these products release acrylonitrile to the environment is not reported. Fumigant formulations containing acrylonitrile were once used as pest control for residential buildings, tobacco, grains, and nuts (Davis et al., 1973). However, today fumigants containing acrylonitrile are no longer in use.

### 5.5.4 Conclusions

The major sources of acrylonitrile emissions in the U.S. are monomer and polymer production facilities. The estimated acrylonitrile emissions from these facilities are shown below:

| Production facility | Estimated Acrylonitrile Emissions (Suta, 1982) (Mg/yr) |
|---|---|
| Monomer | 802 |
| ABS-SAN resin | 1424 |
| Acrylic and modacrylic fiber | 1276 |
| Nitrile elastomer | 295 |
| Adiponitrile | 59.0 |
|  | 3856 |

Although the relative importance of other potential sources of acrylonitrile emissions is difficult to assess, these emissions are believed to be small relative to monomer and polymer production.

# 6. ENVIRONMENTAL FATE, TRANSPORT, AND DISTRIBUTION

The environmental fate of acrylonitrile in air, water, and soil is discussed in the following sections. The discussion is based on only a few studies that have been conducted in the field.

## 6.1 ATMOSPHERIC FATE, PERSISTENCE, AND TRANSPORT

Very few studies have been conducted to investigate the fate of acrylonitrile under atmospheric conditions. Based on the similarity of the physical and chemical properties of acrylonitrile and the olefins, however, it is possible to predict the atmospheric fate of acrylonitrile from what is known about that class of compounds. Like other olefins, acrylonitrile is expected to undergo both chemical and photochemical reactions in the atmosphere. These reactions are discussed individually in the following sections.

### 6.1.1 Atmospheric Chemical Reactions

Although no specific references are available, atmospheric oxidation reactions typical of olefins may take place with acrylonitrile. For example, oxygen atoms formed as a result of the photolysis of nitrogen dioxide in the atmosphere usually add to the olefinic double bond. Oxygen atoms react with olefins more rapidly than with other unsaturated aromatic and hydrocarbons. This addition reaction forms an excited epoxide that subsequently decomposes to alkyl and acyl radicals (U.S. EPA, 1979).

Hydroxyl radicals, formed as a result of atmospheric photolysis of nitrous acid and degradation of other free radicals, add to the double bond of the olefins. The rate constant for this addition reaction is about 10 times greater than for the atomic oxygen olefin reaction (Morris and Niki, 1971).

Atmospheric ozone is formed in significant quantities when nitrogen dioxide levels in the atmosphere are about 25 times greater than nitrogen monoxide levels. Ozone, while not as strong an oxidizing agent as O• or •OH radicals, reacts with olefins at appreciable rates when ozone concentrations reach or exceed 0.25 ppm. Ozone adds to the olefinic double bond forming an aldehyde and a diradical. The diradical may further decompose or may participate in reactions with $O_2$, $NO_2$, and NO.

### 6.1.2 Photochemical Reactions

The photochemistry of acrylonitrile vapor at 213.9 nm was studied by Gandini and Hackett (1978). The photolysis was shown to proceed via two molecular elimination pathways, one yielding acetylene and hydrogen cyanide and the other yielding cyanoacetylene and hydrogen. The quantum yields for the two processes were determined to be 0.50 and 0.31, respectively. In the presence of such photosensitizers as xanthene, triphenylene, benzophenone, acetophenone, fluorenone, and dibromoanthracene, the major product of photolysis of acrylonitrile in solution was shown to be 1,2-dicyanocyclobutane (Gale, 1971; Hosaka and Wakamatsu, 1968). The dicyanocyclobutane is not very stable, however, and it is unlikely that the reaction will proceed in the gas phase.

### 6.1.3 Atmospheric Persistence and Transport

Only one study that experimentally investigated the atmospheric persistence of acrylonitrile is available. Joshi (1977, cited in Suta, 1979) estimated the atmospheric half-life of acrylonitrile to be 9 to 10 hours. An atmospheric half-life of 9-10 hours is sufficiently long for aerial transport to play a significant role in the distribution of acrylonitrile in the neighborhood of emission sources. It has been calculated by Suta (1979) that when the average wind speed is 4 meters/second, 86% of the emitted acrylonitrile will survive at 30 km downwind from the source, and 78% will survive at 50 km downwind.

## 6.2 FATE, PERSISTENCE, TRANSPORT, AND BIOACCUMULATION IN AQUEOUS MEDIA

### 6.2.1 Chemical Reactivity in Water

The chemical stability of acrylonitrile in water at different pH's was studied by Going et al. (1979). He spiked distilled water and Mississippi River water with 10 ppm acrylonitrile. Prior to spiking, the pH was adjusted to 4 or 10 or left unadjusted. All samples were stored at room temperature in Teflon-capped vials for 1, 6, and 23 days. There was no indication of sample decomposition in distilled water even after 23 days at any of the tested pH values. The samples in river water showed decomposition on storage. The sample with unaltered pH showed complete decomposition after 6 days. The sample stored at pH 10 showed little decomposition after 6 days but completely decomposed after 23 days. The pH 4 sample showed even less decomposition after 6 days and only 23% decomposition after 23 days. It is not certain whether the decomposition of acrylonitrile is at least partly due to microbial effect. If so, the extremes of pH may have an inhibitory effect on the microorganisms in the river water. Spiking sterilized water with acrylonitrile and monitoring sample decomposition may provide an answer.

Acrylonitrile, if present in surface waters that are used as sources of drinking water, may react with chlorine or hypochlorite during the chlorination step of the treatment process. It has been suggested that the reaction products could be a mixture of $OHCH_2CHClCN$ and $ClCH_2CHOHCN$ (Kondratenko et al., 1971). The presence of detectable levels of acrylonitrile in previously aerated surface water (used as a source of drinking water), however, is not very likely because of rapid volatilization.

### 6.2.2 Photochemical Reaction in Water

Another mode of acrylonitrile degradation may be photochemical reaction in water; however, little is known regarding the photochemistry of acrylonitrile in water in the concentration region likely to be present in natural water bodies.

### 6.2.3 Degradation of Acrylonitrile by Microorganisms

Limited data suggest that loss of acrylonitrile from water systems via biological degradation can be expected. Both aerobic and anaerobic microorganisms are capable of degrading acrylonitrile, especially acclimated microorganisms. The breakdown products of aerobic microorganisms may include ammonia and acrylic acid (Mills and Stack, 1955), followed by nitrification of ammonia (Chekhovskaya et al., 1966). The latter authors found that acrylonitrile at concentrations of 50 ppm or higher may inhibit nitrification.

Mills and Stack (1955) suggested a mechanism for the biological oxidation of acrylonitrile. Using microorganisms from the Kanawha River (WV) that had been acclimated with acrylonitrile for 27 days, the Biological Oxygen Demand (BOD) of acrylonitrile was measured. The rate of aerobic oxidation was quite rapid and reached completion in five days. As shown in Figure 6-1A, about 70% of the acrylonitrile was degraded. From the nitrogen balance data, the authors suggested that the biological oxidation of acrylonitrile proceeds by an enzyme-catalyzed hydrolysis of the nitrile group to acrylic acid and ammonia.

The microbial fate of acrylonitrile in natural water was studied by Cherry et al. (1956). Acrylonitrile (10 ppm) was added to filtered aerated water from the Hackensack River (NJ). Nitrogen and phosphorus nutrients were added to the water. The complete disappearance of acrylonitrile from water took about 20 days. Subsequent redosing with acrylonitrile reduced the degradation time. These results are shown in Figure 6-1B. Similar results were obtained at 25 and

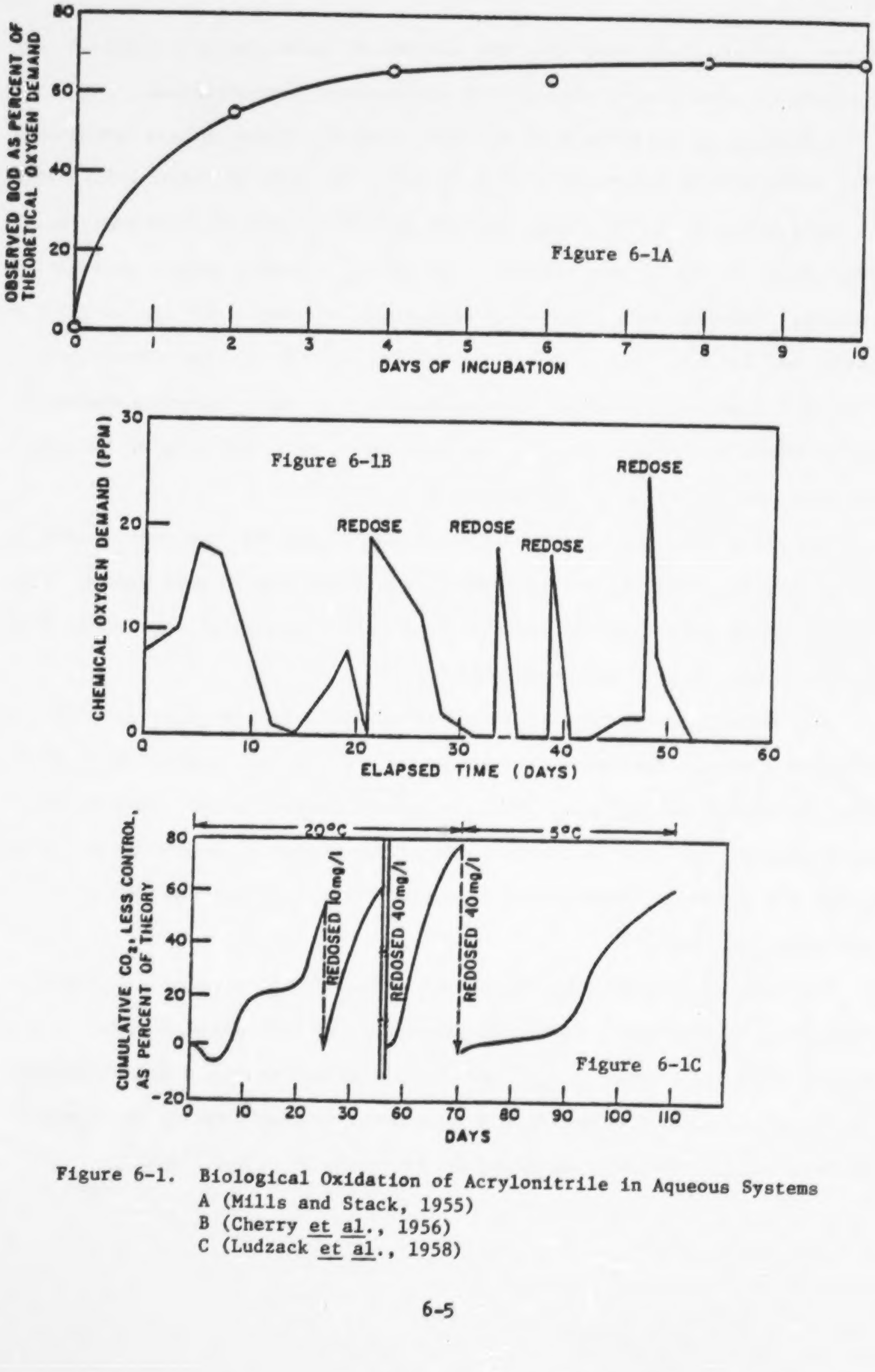


Figure 6-1. Biological Oxidation of Acrylonitrile in Aqueous Systems
A (Mills and Stack, 1955)
B (Cherry _et al_., 1956)
C (Ludzack _et al_., 1958)

50 ppm acrylonitrile; that is, the acclimated microorganisms degraded the acrylonitrile more rapidly than did the unacclimated microorganisms.

Ludzack et al. (1958) also found similar results. These authors spiked Ohio River water with 10 ppm acrylonitrile at 20°C. As shown in Figure 6-1C, there was a lag period of about a week, followed by several days of rapid degradation after which a plateau was reached. By day 22, another period of activity occurred. Redosing this water with acrylonitrile produced no lag period and plateau but produced rapid degradation of acrylonitrile by the already acclimated microorganisms. Evidently the degradation rate was temperature dependent; when a sample was redosed at 5°C, the degradation rate was found to be slower than that seen in the sample redosed at 20°C.

Ludzack et al. (1958) noted that acrylonitrile was degraded more rapidly by microorganisms in Ohio River water than by microorganisms in aged sewage. They also found that acrylonitrile was more resistant to biological degradation than aceto-, adipo-, benzo-, and lacto-nitriles.

The aerobic degradation of acrylonitrile in water can also proceed via activated sludge. Experiments conducted by Dow Chemical Company (NAS, 1975, cited in Miller and Villaume, 1978) indicated almost complete degradation of acrylonitrile to ammonia in 20 days. The effectiveness of acclimated activated sludge for the rapid degradation of acrylonitrile in water was also shown by Ludzack et al. (1961).

The effectiveness of activated sludge systems for almost complete biodegradation of acrylonitrile in industrial wastewater was also shown by Kincannon and Stover (1981) and Freeman et al. (1981). The latter authors demonstrated that the residue level of acrylonitrile in wastewater treatment basins after aerated activated sludge treatment was below the detection limit (<0.1 ppm).

Kato and Yamamura (1976) discovered that aerobic microorganisms of the genus Nocardia were capable of degradation of cyanides and nitriles. More than 90% of the acrylonitrile was degraded by these microorganisms.

The preceeding studies show that acrylonitrile can be degraded aerobically. Ludzack et al. (1961), Lank (1969, cited in Miller and Villaume, 1978), and Hovious et al. (1973, cited in Miller and Villaume, 1978) studied acrylonitrile degradation under anaerobic conditions. Lank (1969, cited in Miller and Villaume, 1978) found that acrylonitrile at a concentration of 10 ppm could be treated by anaerobic digestion. Hovious et al. (1973, cited in Miller and Villaume, 1978), however, determined that, even at a concentration of 50 ppm, acrylonitrile was inhibitory to some anaerobes. The inhibition was not complete, so some residual activity remained. Acrylonitrile's inhibition of anaerobic digestion by microorganisms was also confirmed by Ludzack et al. (1961). These authors recommended that the anaerobic digestion should not be used for treatment of acrylonitrile-containing water.

### 6.2.4 Bioaccumulation in Water

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic organisms. It is important to determine the BCF for acrylonitrile in aquatic organisms in order to evaluate the levels of human intake of acrylonitrile from this source and also to assess ecological effects. There are several theoretical correlation equations that have been established to relate BCF to either the octanol-water partition coefficient or the water solubility of the chemical. These equations are given below:

$$\log BCF = 0.76 \log K_{ow} - 0.23$$ (Veith et al., 1979)

$$\log BCF = 0.542 \log K_{ow} + 0.124$$ (Neely et al., 1974)

$$\log BCF = -0.508 \log S + 3.41$$ (Chiou et al., 1977)

where $K_{ow}$ = partition coefficient of the chemical between octanol and water, and S = water solubility of the chemical expressed in μmol/l.

The above equation of Veith et al. (1979) is applicable for the whole fish, whereas the equations of Neely et al. (1974) and Chiou et al. (1977) are applicable for fish muscle only.

If the values for $K_{ow}$ and S for acrylonitrile are assumed to be 0.12 (Leo et al., 1971) and $1.385 \times 10^6$ μmol/l (Groet, 1978), respectively, the theoretical values for BCF can be calculated to be 0.1 (equation of Veith et al., 1979) for the whole fish and 0.4 (equation of Neely et al., 1974) and 2.0 (equation of Chiou et al., 1977) for fish muscle.

U.S. EPA (1978a) experimentally measured the steady-state BCF for acrylonitrile in bluegills containing about 4.8% lipids. The experimental value was 48. The BCF for lipid-soluble compounds is proportional to percent lipids (U.S. EPA, 1979). The weighted average lipid content in all the aquatic foods consumed by an individual in the United States was calculated to be 3.0% (U.S. EPA, 1979). An adjustment factor of 3.0/4.8 = 0.625 was used to adjust the measured BCF from the 4.8% lipid of the bluegill to the 3.0% lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for acrylonitrile in the edible portion of all aquatic organisms consumed by an individual in the United States was calculated to be 48 x 0.625 = 30 (U.S. EPA, 1980b).

It can be concluded from the above discussions that the experimental BCF for acrylonitrile in consumable aquatic foods is about two orders of magnitude higher than the calculated value.

### 6.2.5 Transport in Water

Few studies are available that investigate the transport of acrylonitrile in water other than the river water studies discussed previously. The partial vapor pressure of acrylonitrile in its water azeotrope is 80 mm Hg at 20°C (Miller and Villaume, 1978); this pressure is significant enough to cause evaporation of acrylonitrile from water. Using the method of Dilling (1977), the half-life of evaporation of acrylonitrile from water with an assumed depth of 1 meter can be calculated to be 795 minutes. It should be mentioned, however, that no experimental data are available to demonstrate this transport possibility and as discussed in Section 3.4.8 the calculated evaporative half-life may not be accurate for acrylonitrile in natural aquatic systems.

The removal of acrylonitrile from an aquatic system will take place through various chemical (photochemical, oxidative etc.), biological and physical (evaporative, adsorptive etc.) processes as discussed in subsections 6.2.1, 6.2.2, 6.2.3 and this subsection. The percent of acrylonitrile removal through each of the processes will depend on the characteristics of the aquatic system. For example, in biologically active systems <0.1% of the acrylonitrile was found to be removed from the aquatic media due to volatilization (Freeman et al., 1980; Kincannon and Stover, 1981). The calculated half-life value given above is only for the fraction of acrylonitrile that is removed from the water by the evaporative route.

The observations from the two studies of accidental spills of acrylonitrile can be used to provide further insight into the transport of acrylonitrile from other media into water (see Section 7.3.4). Both these spill incidents show that it is possible to transport acrylonitrile from contaminated land to surface waters (since the medium of land to water transfer was percolated water). In the absence of evaporative effect, acrylonitrile can be expected to have a long persistence in water. The bacterial decomposition of acrylonitrile in soil will probably be of little importance in cases of spills because of the toxic effect of the large spills on the bacteria. If the spill occurs during winter, the low temperature of soil will further decrease the influence of biodegradation.

## 6.3 FATE, PERSISTENCE, AND TRANSPORT IN SOIL

Few data are available on this subject. Acrylonitrile can be degraded by soil fungi (Giacin *et al.*, 1973). Fungi capable of acrylonitrile biodegradation included *Penicillium*, *Aspergillus*, and *Cladosporium* species (Giacin *et al.*, 1973). The products of decomposition were probably carbon dioxide and ammonia. Although other microorganisms slowly degraded acrylonitrile, best results were obtained with soil fungi. The microbe *Nocardia rhodochrous* LL100-21 slowly degraded acrylonitrile, but the rate of degradation increased with added acetate (DiGeronimo and Antoine, 1976).

# 7. ENVIRONMENTAL LEVELS AND EXPOSURE

## 7.1 ENVIRONMENTAL LEVELS

Acrylonitrile levels in occupational atmospheres (Marrs *et al.*, 1978; Sakurai *et al.*, 1978; Martin, 1978) and in industrial point sources (Hughes and Horn, 1977; Hollingsed, 1978, cited in Miller and Villaume, 1978; Suta, 1979) have been determined. One published study conducted by Midwest Research Institute (Going *et al.*, 1979) has determined environmental levels of acrylonitrile. This study was designed to determine the levels of acrylonitrile in ambient air samples, surface waters, and soils and sediments around acrylonitrile and acrylonitrile polymer manufacturing facilities.

PEDCo Environmental has recently completed a study monitoring levels of AN near 4 acrylonitrile producing or consuming plants. During this study, quantifiable amounts of ACN (>2.5 ppb) were found in the vicinity of each of the plants that were tested. Preliminary results indicate acrylonitrile to have been measured at all four locations and, in general, levels around user plants were greater than around producing plants.

### 7.1.1 Atmospheric Levels of Acrylonitrile Around Its Major Production and Usage Facilities

The atmospheric levels of acrylonitrile around manufacturing facilities are given in Table 7-1. The samples were collected by passing the air through activated carbon over a period of approximately 24 hours for all stations, except Monsanto in Decatur, Alabama, where two 24-hour samples were collected. The acrylonitrile from the activated carbon was desorbed by carbon disulfide and analyzed by gas chromatography with a flame ionization detector. The average recovery of acrylonitrile determined with spiked samples was 63% (Going *et al.*, 1979).

Table 7-1. Atmospheric Monitoring Data for Acrylonitrile (Going *et al.*, 1979)

| Site | Product | Distance (km) of Collection from Plant[a] | No. of Samples Collected[b] | Concentration of Acrylonitrile ($\mu g/m^3$) | | |
|---|---|---|---|---|---|---|
| | | | | High | Low | Maximal Average[c] |
| American Cyanamid, New Orleans, LA | Acrylonitrile | 0.6-1.8 | 5 | 13.6 | <0.1 | 2.6 |
| American Cyanamid, Linden, NJ | Acrylonitrile | 0.5-1.8 | 6 | 15.9 | <0.1 | 3.2 |
| Monsanto, Texas City, TX | Acrylonitrile | 0.7-2.6 | 7 | 8.9 | <0.3 | 3.2 |
| Monsanto, Decatur, AL | Acrylic and Modacrylic Fibers | 1.3-5.0 | 5 | 4.2 | <0.1 | 1.2 |
| DuPont, Lugoff, SC | Acrylic Fibers | 0.7-2.2 | 8 | 1.1 | <0.1 | 0.3 |
| DuPont, Waynesboro, VA | Acrylic and Modacrylic Fibers | 0.3-0.9 | 6 | 7.0 | <0.2 | 1.3 |
| Borg-Warner, Washington, WV | ABS/SAN Resins | 0.5-1.3 | 4 | 325.0 | <0.2 | 84.0 |
| Goodrich, Louisville, KY | Nitrile Elastomers > ABS/SAN Resin | 0.4-3.0 | 4 | 4.3 | <0.2 | 1.2 |
| Monsanto, Addyston, OH | ABS/SAN Resins | 0.3-1.0 | 5 | 1.1 | <0.2 | 0.5 |
| Uniroyal, Painesville, OH | Nitrile Elastomers | 0.3-0.9 | 5 | 3.1 | <0.1 | 1.0 |
| Vistron, Lima, OH | Acrylonitrile > Acrylamide | 0.2-0.6 | 5 | 148.0 | <0.2 | 34.4 |

[a] The distances of sample collection points are obtained from Suta (1979).

[b] Sometimes replicate samples were collected from the same sampling point.

[c] See text for explanation.

The values for maximal average concentrations given in Table 7-1 have been derived in the following manner: the sum of all the determined concentrations has been divided by the number of determinations. When the determined concentrations were less than the detection limit, the data used for averaging were the values at the detection limit.

The highest individual concentration from this monitoring data was 325 $\mu g/m^3$ and the lowest was <0.1 $\mu g/m^3$ (Going et al., 1979). The recorded concentrations depend greatly on the meteorological conditions, the production stage within the plant at the time of sampling, and the presence of emission control devices in the plant. This is reflected in the high maximal average acrylonitrile level (84 $\mu g/m^3$) in one plant and a low level (0.5 $\mu g/m^3$) in another plant, even though both produced ABS/SAN resins. Such high and low atmospheric levels of acrylonitrile are also reflected in the data for other plant emissions, all manufacturing the same acrylonitrile (see Table 7-1). A comparison of the experimental monitoring data (Going et al., 1979) with the dispersion modeling data of Suta (1979) is given in Table 7-2.

The chemical analysis technique used for generation of the data presented in Table 7-2 has since been improved. The extraction of the collected acrylonitrile was performed for the monitoring data using carbon disulfide. The present accepted extraction procedure uses a mixture of carbon disulfide and acetone (49:1) which improves recovery (95%) (Gagnon, 1979) over that of carbon disulfide alone (60-75%) (Silverstein, 1977).

A comparison of the dispersion modeling data (Suta, 1979) with the actual monitoring data (Going et al., 1979) shows that, although the difference between the experimental concentrations and the concentrations derived from dispersion modeling (Suta, 1979), on the average, was about 20%, 90% of the individual values had much higher variations. In many instances, the agreement between the

Table 7-2. Comparison of Monitoring and Dispersion Modeling Data (Suta, 1979)

| Plant/Location | Distances (km) | Acrylonitrile Concentration ($\mu g/m^3$) Monitoring[b] | Acrylonitrile Concentration ($\mu g/m^3$) Dispersion Modeling[c] |
|---|---|---|---|
| American Cyanamid New Orleans, LA | 0.50-0.99 | 4.3 | 4.3 |
| | 1.00-1.49 | 0.1 | 2.5 |
| | 1.50-1.99 | 0.1 | 1.9 |
| American Cyanamid Linden, NJ | 0.50-0.99 | 0.5 | [d] |
| | 1.00-1.49 | 6.0 | [d] |
| | 1.50-1.99 | 0.7 | [d] |
| Monsanto Texas City, TX | 0.50-0.99 | 2.4 | 6.5 |
| | 1.00-1.49 | nd | - |
| | 1.50-1.99 | 3.8 | 2.5 |
| | 2.00-2.49 | 0.9 | 1.8 |
| | 2.50-2.99 | 5.2 | 1.3 |
| Monsanto Decatur, AL | 1.00-1.49 | 1.2 | 21.0 |
| | 1.50-1.99 | 2.3 | 9.3 |
| | 2.00-2.50 | 0.8 | 7.2 |
| | 2.50-4.99 | nd | - |
| | 5.00-5.49 | 0.2 | 3.1 |
| DuPont-May Camden, NJ | 0.50-0.99 | 0.7 | 7.4 |
| | 1.00-1.49 | 0.3 | 2.3 |
| | 1.50-1.99 | 0.1 | 1.3 |
| | 2.00-1.50 | 0.2 | 1.1 |
| DuPont Waynesboro, VA | 0.30-0.49 | 3.6 | 5.2 |
| | 0.50-0.99 | 0.2 | 3.4 |
| Borg-Warner Washington, WV | 0.50-0.99 | 157.6 | 42.1 |
| | 1.00-1.49 | 0.3 | 30.4 |
| B.F. Goodrich Louisville, KY | 0.30-0.49 | 2.3 | 7.2 |
| | 0.50-1.99 | nd | - |
| | 2.00-2.49 | 0.2 | 1.7 |
| | 2.50-2.99 | nd | - |
| | 3.00-3.49 | 0.2 | 1.0 |
| Monsanto Addyston, OH | 0.30-0.49 | 0.2 | 5.9 |
| | 0.50-0.99 | 0.4 | 3.9 |
| | 1.00-1.49 | 1.1 | 2.8 |
| Uniroyal Painesville, OH | 0.30-0.49 | 1.3 | 1.2 |
| | 0.50-0.99 | 0.7 | 0.9 |
| Vistron Lima, OH | 0.30-0.49 | 43.4 | 10.6 |
| | 0.50-0.99 | 0.2 | 5.2 |

nd = no data

[a] Estimated distance from the acrylonitrile production within the plant.

[b] Average of all monitoring stations within the indicated distances.

[c] Estimated concentrations at the midpoint of the distances.

[d] Dispersion modeling estimates were not made for acrylamide plants.

two was poor. Therefore, the need for more experimental monitoring data cannot be overemphasized.

It is interesting to note that the atmospheric acrylonitrile level near the American Cyanamid Plant in Linden, New Jersey, which produces only acrylamide, is comparable to that near plants that manufacture other products derived from acrylonitrile. In deriving the sources of emissions (see Section 5.5.2), Suta (1979) made the assumption that acrylamide production is a negligible source of acrylonitrile emission.

### 7.1.2 Acrylonitrile Levels in Surface Waters

The acrylonitrile monitoring data for surface waters are given in Table 7-3. These data were obtained by Going _et_ _al_. (1979). Whenever possible, grab samples were collected upstream and downstream of the plant discharge points. In some instances, discharged wastewater from the plants was collected for analysis.

Two complementary techniques, azeotropic distillation and purge-trap, were used for reducing interference and concentrating acrylonitrile in the samples. The method used for quantification was GC with a Hall nitrogen-selective detector. Samples that appeared to contain acrylonitrile were confirmed by GC-MS analysis. Analytical quality assurance was done by spiking and analyzing field samples (Going _et_ _al_., 1979).

The two highest levels of acrylonitrile shown in Table 7-3 were obtained from the Monsanto plant in Decatur, Alabama, and the Uniroyal plant in Painesville, Ohio. These values were high, however, because these samples represent discharged wastewater prior to adequate dilution in surface water. The high acrylonitrile content in the discharged wastewaters is an indication that an effective control of these wastewaters is necessary to minimize the pollution of surface waters.

Table 7-3. Acrylonitrile Monitoring Data for Surface Waters (Going *et al*., 1979)

| Site | Product | Concentration in water, µg/l | |
|---|---|---|---|
| | | High | Low |
| Mississippi River near American Cyanamid, New Orleans, LA | Acrylonitrile | n.d.[a] | <0.1 |
| Arthur Hill near American Cyanamid, Linden, NJ | Acrylamide | 0.8 | n.d. |
| Texas City Ship Channel near Monsanto, Texas City, TX | Acrylonitrile | 0.4 | <0.1 |
| Tennessee River near Monsanto, Decatur, AL | Acrylic and modacrylic fibers | 3500 | <0.1 |
| Wateree River near DuPont, Lugoff, SC | Acrylic fibers | 19.7 | <0.1 |
| South River and Discharge Point at DuPont, Waynesboro, VA | Acrylic and modacylic fibers | n.d. | <1.3 |
| Ohio River near Borg-Warner, Washington, WV | ABS/SAN resins | 1.9 | 1.4 |
| Ohio River near B.F. Goodrich, Louisville, KY | Nitrile elastomers and ABS/SAN resins | 2.0 | <1.4 |
| Ohio River near Monsanto, Addyston, OH | ABS/SAN resins | 8.0 | <1.4 |
| Influent and effluent from treatment facilities from wastewater at Uniroyal, Painesville, OH | Nitrile elastomers | 4300 | 9.3 |
| Discharge point at Ottawa River at Vistron, Lima, OH | Acrylonitrile and acrylamide | n.d. | <1.3 |

[a] n.d. = not determined

## 7.2 ACRYLONITRILE LEVELS IN SOILS AND SEDIMENTS

The environmental levels of acrylonitrile in a few soil and sediment samples are shown in Tables 7-4 and 7-5. These data were obtained from the investigations of Going et al. (1979).

The soil samples were collected from the air sampling locations. The collection of sediment samples was restricted by the accessibility of the sediments from the waterbody. The methods of analyses were the same for both soil and sediment samples. The sediments free from excess water and the soil samples were ultrasonically agitated with water. In most analyses, the water extracts were directly injected into the gas chromatograph equipped with a Hall nitrogen-selective detector. One sample each from soil and sediment was further purified and concentrated by the purge-trap technique before GC injection. The recoveries of acrylonitrile from the samples were not determined.

It is obvious from Tables 7-4 and 7-5 that, with the exception of one soil sample, the level of acrylonitrile in all other samples was below the detection limit of the method used. This may be expected in view of the relatively high water solubility and high volatility of acrylonitrile. The detection limit, however, could have been lowered either with the purge-trap or azeotropic distillation of the water extract.

## 7.3 ENVIRONMENTAL EXPOSURE

Population exposure from environmental acrylonitrile emissions can take place through four principal sources: (1) industrial emissions in air; (2) drinking water; (3) consumed foods; and (4) spillage during transportation. The exposure from each of these sources is discussed below.

Table 7-4. Acrylonitrile Monitoring Data for Sediments (Going et al., 1979)

| Site | Concentration (μg/kg)[a] |
|---|---|
| Mississippi River near American Cyanamid, New Orleans, LA | <0.5 |
| Tennessee River near Monsanto, Decatur, AL | <50 |
| Wateree River near DuPont, Lugoff, SC | <50 |
| South River near DuPont, Waynesboro, WV | <50 |

[a] These figures were the lowest detection limit

Table 7-5. Acrylonitrile Monitoring Data for Soils (Going et al., 1979)

| Site | Concentration (μg/kg)[a] |
|---|---|
| American Cyanamid, New Orleans, LA | ≈0.5 |
| American Cyanamid, Linden, NJ | <50 |
| Monsanto, Texas City, TX | <100 |
| Monsanto, Decatur, AL | <50 |
| DuPont, Lugoff, SC | <50 |
| DuPont, Waynesboro, VA | <50 |
| Borg-Werner, Washington, WV | <50 |
| B.F. Goodrich, Louisville, KY | <400 |
| Monsanto, Addyston, OH | <400 |
| Uniroyal, Painesville, OH | <400 |
| Vistron, Lima, OH | <100 |

[a] These figures were the lowest detection limit

### 7.3.1 Exposure From Air Polluted by Industrial Sources

The total number of people expected to be exposed to different levels of acrylonitrile concentrations from different industrial sources was calculated by Suta (1979) and is given in Table 7-6.

The estimated values were derived on the basis of a dispersion modeling from the emitted acrylonitrile concentration values and estimated population density around the plants. The estimated exposure values were determined for people residing within 10 concentric rings (of various radii ranging from 0 to 30 km) about each plant.

The estimated exposures in Table 7-6 are somewhat underestimated for two reasons. First, the exposures beyond 30 km were not included in all the calculations. Second, the exposure from acrylamide plant emissions was ignored, even though the experimental data from Going _et al_. (1979) indicated such exposures might be significant.

### 7.3.2 Exposure From Drinking Water

Trace amounts of acrylonitrile have been detected in drinking water (Kopfler _et al_., 1976), although the amount was not quantitated. In the absence of such data, it is impossible to evaluate the human intake from this source.

### 7.3.3 Exposure From Foods

The three possible sources of acrylonitrile exposure from foods are (a) fish and shellfish; (b) food containers and packaging materials; and (c) foods fumigated with acrylonitrile-containing fumigants.

Edible aquatic organisms may bioconcentrate acrylonitrile from contaminated waters. The weighted average bioconcentration factor for acrylonitrile in the edible portion of all aquatic organisms consumed by Americans has been calculated to be 30 (U.S. EPA, 1980b).

Table 7-6.

Estimates of Population Exposures to Atmospheric Acrylonitrle from Specific Emission Source Categories (Suta, 1982)

| Average Annual AN Concentration[a] (µg/m) | AN Monomer | ABS/SAN Resins | Acrylic and Modacrylic Fibers | Nitrile Elastomer | Adiponitrile |
|---|---|---|---|---|---|
| 15.0 - 19.9 | | | | | |
| 10.0 - 14.9 | | | | | |
| 5.0 - 9.9 | | 2,700 | | | |
| 1.0 4.9 | 4,900 | 14,000 | 390 | 18,000 | |
| 0.50 - 0.99 | 36,000 | 53,000 | 5,000 | 46,000 | |
| 0.10 - 0.49 | 260,000 | 238,000 | 140,000 | 421,000 | 22,000 |
| 0.050 - 0.099 | 720,000 | 382,000 | 440,000 | 425,000 | 20,000 |
| 0.010 - 0.049 | 1,602,000 | 1,407,000 | 320,000 | 1,254,000 | 78,000 |
| 0 005 - 0.009 | 8,000[b] | 456,000[b] | 26,000[b] | 265,000[b] | 0[b] |
| 0.001 - 0.004 | 28,000[b] | 1,701,000[b] | 15,000[b] | 292,000[b] | 0[b] |
| Total People Exposed | 2,659,000[b] | 4,254,000[b] | 946,000[b] | 2,721,000[b] | 120,000[b] |

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[a] To convert for µg/m$^3$ to ppb, multiply by 0.46.

[b] Exposures in these ranges are underestimates because calculations were only made for exposures within 30 km of each plant.

The polymers and copolymers containing residual acrylonitrile monomer could migrate from the food-contact items to the food itself. The amount of migration depends on the residual monomer content in the polymer or copolymer, the time of storage, and the temperature of storage. The effects of these factors on acrylonitrile migration are shown in Table 7-7.

Since the monomer migration is substantial from ABS/SAN resins to ethanol, the FDA currently does not permit the use of these containers for alcohol and carbonated beverages. Gawell (1979), using SAN bottles containing 3 to 5 ppm residual monomer, showed, however, that the migration in some samples of beer and soft drinks amounted to < 0.005 mg/kg. The author did not specify the storage conditions. FDA has determined that the migration of monomer from SAN resin containers (3.3 ppm residual acrylonitrile) to the beverages could be as high as 14 ppb (Flood, 1980) after 96 days contact at 120°F.

Under FDA regulations, copolymers of acrylonitrile listed in Table 7-8 are permitted in food-contact applications including food packaging, such as for luncheon meats, peanut butter, margarines, fruit juices, and vegetable oils. Some acrylonitrile exposure may result from these sources, although it is difficult to quantify the amount. FDA has determined that the migration of the monomer from the containers to vegetable oil and margarine could be as high as 37 ppb (Flood, 1980).

Foods that have been fumigated with acrylonitrile-containing fumigants present a risk of acrylonitrile exposure. Fumigants containing acrylonitrile for grain pest control have been voluntarily withdrawn from the market. Other foods, such as walnuts, are no longer fumigated with acrylonitrile-containing fumigants. The residue level of acrylonitrile in walnuts that have been fumigated may range from 7.5-17.5 ppm after 2 days to 0-8.5 ppm after 38 days (Berck, 1960). Finally, acrylonitrile has been used as a fumigant for stored tobacco.

Table 7-7. Acrylonitrile Migration Under Different Storage Conditions

| Migrating Solvent | Nature of Plastic | Residual Monomer Content of Plastic (ppm) | Time of Storage (months) | Temp. of Storage (°C) | Acrylonitrile Found (ppm) | Reference |
|---|---|---|---|---|---|---|
| 3% acetic acid | SAN | 7 | 1 | 49 | 0.013 | Brown _et_ _al_., 1978 |
| | | | 2 | 49 | 0.017 | Brown _et_ _al_., 1978 |
| | | | 3 | 49 | 0.022 | Brown _et_ _al_., 1978 |
| | | | 4 | 49 | 0.028 | McNeal _et_ _al_., 1979 |
| | | | 5 | 49 | 0.038 | Brown _et_ _al_., 1978 |
| 3% acetic acid | SAN | 3 | 2 | 49 | 0.012 | McNeal _et_ _al_., 1979 |
| | | | 2 | 66 | 0.023 | McNeal _et_ _al_., 1979 |
| 3% acetic acid | ABS | 24 | 1 | 49 | 0.283 | Brown _et_ _al_., 1978 |
| | | | 2 | 49 | 0.557 | Brown _et_ _al_., 1978 |
| | | | 3 | 49 | 0.723 | Brown _et_ _al_., 1978 |
| | | | 5 | 49 | 0.920 | Brown _et_ _al_., 1978 |
| 8% ethanol | SAN | 10 | ? | 49 | 0.078 | McNeal _et_ _al_., 1979 |
| | | 25 | ? | 49 | 0.126 | McNeal _et_ _al_., 1979 |

Table 7-8. Amounts of Various Acrylonitrile Copolymers Used in Food-Contact Applications (Troxell, 1980)

| Copolymer | Use[a] | Amount used in 1977 (millions of pounds) |
|---|---|---|
| ABS Resins | Refrigerator/freezer linings | 125 |
| ABS Resins | Small appliances (motor housings, bases for blenders and can openers, etc.) | 10-15 |
| ABS Resins | Packaging (sheet and film for blister packs) | 15 |
| ABS Resins | Margarine tubs | <1 |
| SAN Resins | Drinking tumblers, blenders, jars, components of appliances, etc. | ≈50 |
| Nitrile Elastomers/ Latexes | Hoses and paper coating | 7-14 |
| High Nitrile Resins | Mostly vegetable oil bottles | <10 |
| Polyvinylidine chloride - Acrylonitrile Resin | Cellophane > paperboard | ≈20 |

[a] Some of the applications may result in incidental food contact.

Guerin *et al*. (1974) have qualitatively determined the presence of acrylonitrile in tobacco smoke, and the amount has been determined to be 1-2 mg per U.S. cigarette smoked (IARC, 1979). It is not clear whether the source of acrylonitrile is from fumigation of the tobacco or from the combustion process itself.

### 7.3.4 Exposure From Spillage during Transportation

The expected numbers of people in urban/rural areas exposed annually from spills during transportation of acrylonitrile by three modes of transportation were calculated as follows: barge, 0.008/0.004; truck, 0.010/0.002; and rail, 0.16/0.016 (Miller and Villaume, 1978). These figures represent the cases in which the entire commodity is transported by one mode. It can be concluded that transportation of acrylonitrile by rail (40.4% of overall shipment) poses the greatest hazard and by barge (1.7% of the overall shipment) the least hazard.

The preceding estimate was based on exposure due to ignition of the spilled area and toxic exposure from contaminated surface water due to spill (see Section 5.5.2). However, contamination of the ground water and the subsequent exposure from usage of the water as a source of drinking water was not considered in the calculation.

Two recent cases of accidental spills leading to ground water contamination show the possibility of population exposure from this source. A spill of 36,000 gallons of acrylonitrile onto farmland (Gilford, Inc., 2/22/77) caused contamination of the nearby groundwater and a creek (Miller and Villaume, 1978). Evidently, the acrylonitrile percolated through the soil into the groundwater and the creek. For several months after the spill, the concentration of acrylonitrile in the groundwater increased after it rained (Miller and Villaume, 1978).

Another spill of 20,000 gallons of acrylonitrile (near Mapleton, IL, 12/23/77) caused similar contamination of the groundwater and creeks located near the spill (Miller and Villaume, 1978). Monitoring data from five wells, all located within 100 feet from the spill site, showed high levels of acrylonitrile in the water (46 mg/l to 3520 mg/l) at the end of 108 days (Miller and Villaume, 1978). Acrylonitrile levels started decreasing about 170 days after the spill, but acrylonitrile had not completely disappeared from the well water even after 351 days. Nine additional wells, located at an average distance of about 1000 feet from the spill site, showed no trace of acrylonitrile. Tap water at six nearby residences (an average distance of 1150 feet from the spill site) contained no acrylonitrile. A little Marsh Creek located about 750 feet from the site of the spill showed 32 mg/l of acrylonitrile at the end of 58 days after the spill, but acrylonitrile finally disappeared after about 108 days.

### 7.3.5 Exposure From Thermal Degradation

Thermal decomposition of polymers containing acrylonitrile is another source of acrylonitrile in the atmosphere. Pyrolysis of the following polymers--polyacrylonitrile (Tsuchiya and Sumi, 1977; Guyot et al., 1978), ABS/SAN resins (Chaigneau and LeMoan, 1974), acrylonitrile-methacrylate copolymers (Guyot et al., 1978), vinyl chloride-acrylonitrile copolymers (Tanaka et al., 1975)--produces hydrogen cyanide and acrylonitrile.

The composition of the gases evolved during pyrolysis and combustion of polymers and copolymers of acrylonitrile depends on a number of factors, including the nature of the polymer, gas composition, gas flow rate, and heating rate of the flame (Sarofim et al., 1973). Pyrolysis prevails at lower temperature and decomposition requires higher temperature (Sarofim et al., 1973). The pyrolysis of SAN bottles with He and air at a flow rate of 1 to 3 l/min and heated at a rate of 5°C/min to 480°C/min produced the following major nitrogen components (Sarofim et al., 1973).

## 7.4 CONCLUSIONS

Most acrylonitrile exposures result from ABS/SAN resin and nitrile elastomer production. The risk for population exposure from the acrylonitrile sources is dependent on a number of factors, including the height of the release point source; that is, the higher release point results in greater dilution of the pollutants at ground level and also spreads them over a larger area. In general, for the same amount of emission and topography, acrylonitrile emission points with lower elevation will result in higher ground level concentrations than elevated acrylonitrile emission points. On the basis of the elevation and other relevant factors, monomer and ABS/SAN resin production results in the highest estimated total risk in terms of exposed population and exposure concentration.

# 8. BIOLOGICAL EFFECTS ON MICROORGANISMS

Loveless *et al*. (1954) studied the effect of acrylonitrile on growth and cell division of yeast (*Saccharomyces cerevisiae*) and bacteria (*Escherichia coli*), as measured by dry weights and cell counts during the logarithmic growth phase. Treatment with 1000 mg/l reduced the growth of *E. coli* but had no effect on cell size; this concentration inhibited growth and division in *S. cerevisiae*. Treated cells were 170% larger than control cells and weighed 52% more.

Acrylonitrile was not toxic to the bacterium *Nocardia rhodochrous* at a concentration of 10,000 mg/l in both (DiGeronimo and Antoine, 1976). This concentration of acrylonitrile supported growth of these bacteria as a sole source of nitrogen, but not as a source of carbon.

Acrylonitrile has been shown to be inhibitory to anaerobic bacteria. Ludzack *et al*. (1961) reported that acrylonitrile inhibited gas production by anaerobic digester cultures which were dosed repeatedly with 10 to 40 mg/l acrylonitrile. Hovious *et al*. (1973, cited in Miller and Villaume, 1979) found that 50 to 100 mg/l acrylonitrile inhibited gas production by anaerobic methanogenic bacteria in proportion to dose.

Acrylonitrile was used as a fumigant to control mold growth on packaged papads, an Indian bread (Narasimhan *et al*., 1972). Papads with 18 or 20% moisture content were sealed in polyethylene bags, fumigated for 48 hours with 32 or 64 mg/l acrylonitrile, and checked for mold growth after one month. The higher dose prevented mold growth at both moisture levels, whereas the lower dose prevented mold growth only in the 18% moisture papads. The species of molds were not identified.

Some limited information concerning the effects of acrylonitrile on aquatic microorganisms was provided by Cherry *et al*. (1956). Nutrient-enriched and aerated river water was dosed with 10, 25, or 50 mg/l acrylonitrile. Balanced populations of bacteria, diatoms, algae, protozoa, and rotifers developed at 10 and 25 mg/l, whereas fungal species predominated at 50 mg/l.

# 9. BIOLOGICAL EFFECTS ON PLANTS

There is limited information concerning the effects of acrylonitrile on plants.

Garrison (1978) studied the effects of acrylonitrile on cultured seagrass (*Ruppia maritima*). Acrylonitrile was added to the water column to give concentrations ranging from 10 µg/l to 10 g/l. Concentrations greater than 100 mg/l totally inhibited photosynthesis and respiration, as measured by dissolved oxygen changes. Lower concentrations had no effect on these processes. Although all concentrations reduced the growth rate of shoots, the growth rate of roots was stimulated at concentrations below 1 mg/l acrylonitrile.

The effect of acrylonitrile on pea seedlings (*Pisum sativum*) was studied by Burg and Burg (1967), who reported that 0.17 mM (*ca*. 9 ppm) acrylonitrile was "toxic" (undefined effect) and that lower levels showed no effect on seedling elongation.

Fumigant mixtures of acrylonitrile:carbon tetrachloride (1:1) had no adverse effects on seed germination of beans, beets, corn, peas, lettuce, onions, tomatoes, wheat, and oats when seeds were fumigated for 24 to 48 hours at concentrations ranging from 1 to 25 pounds per 1000 cubic feet (Glass and Crosier, 1949, cited in Miller and Villaume, 1978).

When acrylonitrile was added to aerated, nutrient-enriched river water, balanced growth of bacteria, diatoms, algae, protozoa, and rotifers occurred at 10 to 25 mg/l acrylonitrile (Cherry *et al*., 1956). At 50 mg/l, however, fungal growth predominated.

Kihlman (1961) reported that 1 mM (53 ppm) acrylonitrile was not mutagenic to broad bean root tips (*Vicia faba*). The details of this study are given in Section 13.4.

# 10. BIOLOGICAL EFFECTS ON DOMESTIC ANIMALS

No information was found concerning the effects of acrylonitrile on domestic animals other than dogs and cats. The effects of acrylonitrile on these animals are discussed in Section 13.

## 11. BIOLOGICAL EFFECTS ON WILDLIFE

No information was found on the toxicity of acrylonitrile to wildlife other than insects.

### 11.1 INSECTS

Judson et al. (1962) studied the ovicidal effects of acrylonitrile and other chemicals on the eggs of the yellow-fever mosquito (*Aedes aegypti*). Mature eggs were exposed for 24 hours to the vapor of 5 or 10 µl added acrylonitrile in sealed one quart jars (21 to 32°C, 100% humidity) and then placed in deoxygenated water to determine hatchability. The percent mortality at the two treatment levels (4.2 or 8.4 mg/l, by calculation) was 60 and 92%, respectively.

Bond (1963) exposed adult granary weevils (*Sitophilus granarius*) and cadelle larvae (*Tenebroides mauritanicus*) to a series of acrylonitrile fumigant concentrations for an unspecified period. The exposed insects were then divided into three groups, which were kept for 48 hours in an atmosphere of nitrogen, oxygen, or air and then placed in air for 5 days. The dosage (expressed as the product of concentration and exposure time) required to kill 50% of the insects kept in air was 23.0 for *T. mauritanicus* and 5.4 for *S. granarius*. The median lethal concentration cannot be calculated from these values because Bond did not specify the duration of exposure to the fumigant. The results did indicate, however, that oxygen enhanced the toxicity of acrylonitrile and most of the other chemicals tested.

Lindgren et al. (1954) fumigated eight species of insects with a series of acrylonitrile concentrations for 2 or 6 hours. Mortalities were counted 4 days after fumigation. The LD50, LD95, and LD99 values are given in Table 11-1.

Similar toxicity studies were conducted with acrylonitrile by Bond and Buckland (1976, 1978) with several insect species. The duration of exposure,

Table 11-1. Lethal Dose Values for Insects Exposed to Acrylonitrile Fumigation

| Species | Length of Exposure (hours) | Length of Test (days) | LD50 (mg/1) | LD95 (mg/1) | LD99 (mg/1) | Reference |
|---|---|---|---|---|---|---|
| Granary Weevil | 8 | 7 | 0.7 | - | 1.2 | Bond & Buckland, 1976 |
| (*Sitophilus granarius*) | 6 | 4 | 2.0 | 2.9 | - | Lindgren *et al.*, 1954 |
|  | 5 | 7 | 1.4 | - | 2.2 | Bond & Buckland, 1978 |
|  | 2 | 4 | 4.5 | 8.0 | - | Lindgren *et al.*, 1954 |
| Rice Weevil | 6 | 4 | 1.0 | 1.8 | - | Lindgren *et al.*, 1954 |
| (*Sitophilus oryza*) | 2 | 4 | 2.5 | 6.5 | - | Lindgren *et al.*, 1954 |
|  | 24 | 7 | 0.40 | 0.78 | - | Rajendran & Muthu, 1976 |
| Mexican Bean Weevil | 6 | 4 | 1.4 | 2.1 | - | Lindgren *et al.*, 1954 |
| (*Zabrotes pectoralis*) | 2 | 4 | 2.0 | 4.0 | - | Lindgren *et al.*, 1954 |
| Bean Weevil | 6 | 4 | 1.1 | 2.0 | - | Lindgren *et al.*, 1954 |
| (*Acanthosceelides obtectus*) | 2 | 4 | 3.0 | 5.5 | - | Lindgren *et al.*, 1954 |
| Drug-store Beetle | 6 | 4 | 1.7 | 2.5 | - | Lindgren *et al.*, 1954 |
| (*Stegobium paniceum*) | 2 | 4 | 3.0 | 7.0 | - | Lindgren *et al.*, 1954 |
| Confused Flour Beetle | 8 | 7 | 1.9 | - | 2.5 | Bond & Buckland, 1976 |
| (*Tribolium confusum*) | 6 | 4 | 3.0 | 4.9 | - | Lindgren *et al.*, 1954 |
|  | 2 | 4 | 6.5 | 11.0 | - | Lindgren *et al.*, 1954 |
| Pulse Beetle<br>(*Callosobruchus chinensis*) | 24 | 2 | 0.43 | 0.56 | - | Rajendran & Muthu, 1976 |
| Red Flour Beetle | 5 | 7 | 2.6 | - | 4.3 | Bond & Buckland, 1978 |
| (*Tribolium castaneum*) | 24 | 7 | 1.05 | 1.32 | - | Rajendran & Muthu, 1976 |
| Saw-toothed Grain Beetle | 6 | 4 | 1.1 | 2.0 | - | Lindgren *et al.*, 1954 |
| (*Oryzaephilus surinamensis*) | 2 | 4 | 3.5 | 6.5 | - | Lindgren *et al.*, 1954 |
|  | 24 | 7 | 0.76 | 1.27 | - | Rajendran & Muthu, 1976 |
| Lesser Grain Borer | 6 | 4 | 0.8 | 1.4 | - | Lindgren *et al.*, 1954 |
| (*Rhyzopertha dominica*) | 2 | 4 | 2.5 | 4.0 | - | Lindgren *et al.*, 1954 |
|  | 24 | 7 | 0.76 | 1.08 | - | Rajendran & Muthu, 1976 |
| Cadelle | 8 | 7 | 2.8 | - | 6.0 | Bond & Buckland, 1976 |
| (*Tenebroides mauritanicus*) | 5 | 7 | 3.8 | - | 8.4 | Bond & Buckland, 1976 |

time at which mortalities were counted, and the LD50 and LD99 values are given in Table 11-1. Bond and Buckland (1976) found that acrylonitrile alone was more toxic than methyl bromide alone or mixtures of both compounds. Although acrylonitrile is too flammable for use as a fumigant alone, it does enhance the toxicity of non-flammable methyl bromide, especially at low temperatures. Bond and Buckland (1978) showed that fumigation with acrylonitrile and methyl bromide:acrylonitrile mixtures was more effective in atmospheres of 20 to 50% carbon dioxide than in air.

Rajendran and Muthu (1976) also conducted fumigation bioassays with six species of stored product insects. As shown in Table 11-1, the longer exposure period (24 hours) resulted in LD50 and LD95 values that were lower than those reported for the same species by the previously cited workers.

The results presented in Table 11-1 indicate that the concentration of acrylonitrile required to kill 95% or more of test groups of insects is between about 0.5 and 10 mg/l, depending on species and exposure time. This concentration is between 1.3 and 2.6 times the LD50 concentration.

The only other information found concerning effects of acrylonitrile in insects was by Benes and Sram (1969), who evaluated the mutagenic potential of acrylonitrile in fruit flies (Drosophila melanogaster). Details of this study are presented in Section 13.4.1.2.

# 12. BIOLOGICAL EFFECTS ON AQUATIC ORGANISMS

## 12.1 ACUTE TOXICITY

The acute toxicity of acrylonitrile has been determined for several species of marine and freshwater fish and invertebrates.

### 12.1.1 Freshwater Fish

The majority of information concerning the acute toxicity of acrylonitrile to aquatic organisms has been developed with freshwater fish. The most comprehensive study is that of Henderson et al. (1961), who reported median lethal concentration (LC50) values for fathead minnows (Pimephales promelas), bluegill sunfish (Lepomis macrochirus) and guppies (Poecilia reticulata = Lebistes reticulatus). Each bioassay utilized 5 acrylonitrile concentrations in a geometric series and 10 fish per concentration. The test solutions were not renewed during the 96-hour exposure period. LC50 values were calculated by graphical interpolation from mortality data at 24, 48, and 96 hours of exposure. These and other values are presented in Table 12-1.

The 96-hour LC50 values ranged between 11.8 mg/l for the most sensitive species (bluegills) and 33.5 mg/l for the least sensitive species (guppies). The LC50 value decreased by a factor of about 2 between 24 and 96 hours in most tests, indicating that toxicity increased with exposure time. In contrast, toxicity tests with other organic nitriles (lactonitrile, benzonitrile, acetonitrile, adiponitrile, oxydipropionitrile) showed relatively little or no increase in the toxicity of these compounds with longer exposure time. Acrylonitrile LC50 values for fathead minnows were slightly lower in hard water (320 mg/l hardness) than in soft water (20 mg/l hardness), which indicates that acrylonitrile toxicity may increase with higher water hardness. Because confidence intervals for these LC50 values cannot be calculated from the reported data, it is unknown whether the

Table 12-1. Median Lethal Concentration (LC50) Values for Fish Exposed to Acrylonitrile

| Species | Temp. (1°C) | Type Test | Dilution Water | LC50 (mg/1) 24 hr | 48 hr | 72 hr | 96 hr | Reference |
|---|---|---|---|---|---|---|---|---|
| Fathead Minnows[a] (Pimephales promelas) | 25 | S | FW(hard)[f] | 32.7 | 16.7 | - | 14.3 | Henderson et al., 1961 |
| Fathead Minnows[a] (Pimephales promelas) | 25 | S | FW(soft)[g] | 34.3 | 21.5 | 20.5 | 18.1 | Henderson et al., 1961 |
| Fathead Minnows[a] (Pimephales promelas) | 25 | F | FW(soft)[g] | 33.5 | 14.8 | 11.1 | 10.1 | Henderson et al., 1961 |
| Bluegill Sunfish[b] (Lepomis macrochirus) | 25 | S | FW(soft)[g] | 25.5 | 14.3 | - | 11.8 | Henderson et al., 1961 |
| Guppies[c] (Poecilia reticulata) | 25 | S | FW(soft)[g] | 44.6 | 33.5 | - | 33.5 | Henderson et al., 1961 |
| Goldfish (Carassius auratus) | NR | S | FW | - | - | 40 | - | Paulet and Vidal, 1975 |
| Zebrafish (Brachydanio rerio) | 20 | F | FW | - | 15 | - | - | Sloof, 1978 |

Table 12-1. Median Lethal Concentration (LC50) Values for Fish Exposed to Acrylonitrile (cont.)

| Species | Temp. (1°C) | Type Test | Dilution Water | LC50 (mg/l) | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | 24 hr | 48 hr | 72 hr | 96 hr | |
| Minnow (*Phoxinus phoxinus*) | NR | NR | FW | 38.2 | 17.6 | - | - | Marconi and Ionescu, 1974 |
| Carp (*Cyprinus carpio*) | NR | NR | FW | 37.4 | 24.0 | - | - | Marconi and Ionescu, 1974 |
| Rainbow Trout (*Salmo gairdneri*) | NR | NR | FW(hard) | - | 70 | - | - | Jackson and Brown, [illegible] |
| Golden Ide[d] (*Leucisus idus melanotus*) | 20 | S | FW[h] | - | 13,28 | - | - | Juhnke and Luedemann, 1978 |
| Pinfish[e] (*Lagodon rhomboides*) | 13.7-20.4 | S | SW | 24.5 | - | - | - | Daugherty and Garrett, 1951 |

[a] length 50.8-63.5 mm (2-2.5 inches); weight ≈1.5 g

[b] length 38.1-50.8 mm (1.5-2 inches); weight ≈2 g

[c] length 25.4 mm (1 inch); weight ≈0.1 g

[d] length 50-70 mm; weight 1.5 ± 0.3 g

[e] standard length 57-113 mm

[f] hardwater - pH 8.2, alkalinity 320 mg/l; acidity 0 mg/l; hardness 380 mg/l

[g] soft water - pH 7.4, alkalinity 16 mg/l; acidity 2 mg/l; hardness 20 mg/l

[h] pH 7-8; degree of water hardness 15 ± 3° (German "Hartegrad")
S = static exposure; F = flow through exposure; FW = freshwater; SW = seawater; NR = Not reported

difference is statistically significant. It can be concluded, however, that water hardness has little effect on acrylonitrile toxicity.

Henderson et al. (1961) also conducted continuous-flow acrylonitrile bioassays with fathead minnows in soft water. The exposure conditions are described in Section 12.2 and the 24, 48, 72, and 96 hour LC50 values are given in Table 12-1. Comparison of static and continuous-flow LC50 values shows that toxicity is equal at 24 hours, but is greater under continuous-flow conditions after 48 hours. Lower toxicity under static conditions may indicate that acrylonitrile was lost from water through adsorption, volatility, chemical change, fish uptake, or biodegradation.

Renn (1955) exposed bluegill sunfish under static and continuous-flow exposure conditions to 0.38-3.79 mg/l acrylonitrile and found no mortality during a 24-hour exposure period. White crappies (Pomoxis annularis) exposed to 4 acrylonitrile concentrations under continuous-flow conditions began dying after about 2 hours in 90.9 mg/l and after about 8 hours in 68.2 mg/l. No mortality occurred during 24-hour exposure to 37.9 or 22.7 mg/l. The concentrations given here were calculated from Renn's concentration data, which were reported in mg/l nitrogen as acrylonitrile.

The 48-hour LC50 of acrylonitrile to zebrafish (Brachydanio rerio) was determined to be 15 mg/l by Sloof (1978). This bioassay was conducted with 10 fish per concentration in closed 10-liter aquaria under flow-through (6 l/hr) conditions.

Paulet and Vidal (1975) determined a 72-hour LC50 of 40 mg/l for goldfish (Carassius auratus). This bioassay was conducted under static conditions in 12-liter aquaria. No other information was provided.

Bandt (1953) provided some limited information concerning the static toxicity of acrylonitrile to two freshwater fish species, bleak (Ablurnus alburnus) and roach (Rutilus rutilus). Bleak and roach were exposed in groups of one or two fish to 20 to 100 mg/l acrylonitrile for up to 20 days. Although concentrations of 25 mg/l and greater were eventually lethal, the only bleak exposed to 20 mg/l showed no effect after 20 days of exposure. Renn concluded from this limited information that the threshold concentration for prolonged exposure would be about 20 to 25 mg/l. As seen in Section 12.2, this value is much too high.

Juhnke and Luedemann (1978) reported acute toxicity levels of acrylonitrile to golden ide (Leuciscus idus melanotus) that had been determined by using the identical protocol in two different laboratories. The 48-hour LC0, LC50, and LC100 (0, 50, 100% mortality) values determined at each laboratory are given below:

| | 48 Hour Lethal Concentration Values (mg/l) | | |
|---|---|---|---|
| | LC0 | LC50 | LC100 |
| Laboratory 1 | 16 | 28 | 48 |
| Laboratory 2 | 8 | 13 | 20 |

Although these tests presumably were conducted under identical conditions (see Table 12-1), the values reported by laboratory 1 are about twice as high as those reported by laboratory 2. The authors did not discuss the reasons for the different results.

### 12.1.2 Marine Fish

The only report of acrylonitrile toxicity to marine fish is by Daugherty and Garrett (1951). Groups of eight pinfish (Lagodon rhomboides) were acclimated for 22 to 24 hours in 30 liters of aerated seawater at 13.7 to 20.4°C. Acrylonitrile was then added to give 16 concentrations ranging from 0.25 to 60 mg/l. No deaths occurred at 20 mg/l or less, or in controls. All fish died at 30 mg/l, which was the next higher concentration tested. The 24-hour LC50, as determined by graphical interpolation, was 24.5 mg/l. This value is about the same as the 24-hour LC50 values reported for freshwater fish (Table 12-1).

### 12.1.3 Freshwater Invertebrates

Bandt (1953) provided some limited information concerning the static acute toxicity of acrylonitrile to scuds (*Gammarus* sp., a freshwater crustacean). Groups of ten scuds were exposed to 25, 50, or 100 mg/l acrylonitrile for up to 72 hours. All animals exposed to 50 and 100 mg/l were dead by 48 hours. There was no mortality in the group exposed to 25 mg/l for 3 days.

Acrylonitrile may be more toxic to *Daphnia magna*, another species of freshwater crustacea (LeBlanc, 1980). The 24 and 48-hour LC50 values (and 95% confidence interval values) for this species tested under static conditions were 13 (11-15) and 7.6 (6.2-9.2) mg/l, respectively. The "no discernible effect concentration" was 0.78 mg/l.

Randall and Knopp (1980) determined the static 48-hour EC50 (median effective concentration) of acrylonitrile for *D. magna*. This value (and its 95% confidence interval) was 10.95 (9.54-12.56) mg/l, which is in reasonably good agreement with the 48-hour LC50 value reported by LeBlanc (1980). Both of these studies utilized young (< 24 hours old) *D. magna*, and water of similar mean hardness (155-173 mg/l as $CaCO_3$) and temperature (22°C).

### 12.1.4 Marine Invertebrates

Portmann and Wilson (1971, cited in Miller and Villaume, 1978) exposed groups of 8 to 25 brown shrimp (*Crangon crangon*) to serial dilutions of acrylonitrile in aquaria containing 10 gallons (37.85 l) seawater at 15°C. The reported LC50 was 10-33 mg/l.

## 12.2 SUBCHRONIC TOXICITY

There is information concerning the subchronic toxicity of acrylonitrile to several species of freshwater fish and one species of freshwater invertebrate. No information was found for marine fish or invertebrates.

### 12.2.1 Freshwater Fish

Henderson et al. (1961) conducted five replicate subchronic bioassays with fathead minnows under continuous-flow exposure conditions. Groups of 50 fish were exposed to each of 7 acrylonitrile concentrations or to control water (20 mg/l hardness, 25°C) for up to 30 days. Controlled amounts of test solutions were pumped into glass bottles containing 10 fish and 10 liters of water so that a renewal time of 100 minutes was obtained. The fish were fed daily. The mean LC50 values determined for the five replicate bioassays are given below:

| Exposure Time (Days) | 1 | 2 | 3 | 4 | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| LC50 (mg/l) | 33.5 | 14.8 | 11.1 | 10.1 | 8.1 | 6.9 | 5.2 | 4.2 | 3.5 | 2.6 |

The LC50 decreased linearly with exposure time between 15 and 30 days, indicating that the lethal threshold concentration had not been determined at the 30 day mark and that mortalities would have continued to occur at concentrations well below 2.6 mg/l after 30 days.

Cumulative subchronic toxicity was also found by Jackson and Brown (1970, cited in Miller and Villaume, 1978), who exposed rainbow trout to 2 to 200 mg/l acrylonitrile for time periods as long as 100 days. Although the LC50 after 48-hour exposure was 70 mg/l, exposure to 2.2 mg/l for 100 days resulted in 50% mortality.

Very little is known about the mechanisms of acrylonitrile toxicity in fish. Henderson et al. (1961) noted that the first sign of acrylonitrile toxicity in fathead minnows was extreme darkening of the skin, followed in 1 to 3 days by death. They found no cyanide, formed from acrylonitrile, in the exposure water.

In contrast, the more rapid, non-cumulative toxicity of lactonitrile was attributed to the formation of cyanide, which was measured in the exposure water. It is not known whether metabolic formation of cyanide from acrylonitrile occurs in fish, although cyanide or thiocyanate formation has been reported in mammals (Section 13). Toxic action in mammals is attributed primarily to direct effects of acrylonitrile and secondarily to cyanide toxicity. Sloof (1978) studied the effect of acrylonitrile and other chemicals on respiratory activity of rainbow trout. The frequency of respiratory movements was significantly increased after 24-hour exposure to 5 mg/l acrylonitrile under flow-through conditions. This effect was preceded by a temporary, but significant, decrease in breathing rate. The concentration at which this sublethal effect occurred (5 mg/l) was three times lower than the 48-hour LC50 for zebrafish (15 mg/l), determined under similar flow-through exposure conditions.

### 12.2.2 Freshwater Invertebrates

The only information concerning subchronic toxicity of acrylonitrile to aquatic invertebrates was developed with *Daphnia magna* (U.S. EPA, 1978). No adverse effects were found when this invertebrate was exposed over its entire life cycle to as much as 3.6 mg/l acrylonitrile. As mentioned previously (12.1.3), the 48-hour EC50 reported for this species was 7.55 mg/l, which is only about twice as high as the chronic no-observed-effect concentration. In contrast, the 48-hour LC50 was about 32 times higher than the 100-day LC50 for rainbow trout (Jackson and Brown, 1970, cited in Miller and Villaume, 1978) and about six times higher than the 30-day LC50 for fathead minnows (Henderson *et al*., 1961). Although the chronic no-observed-effect concentration has not been determined with any fish species, it is probable that it would be considerably lower than 2 mg/l.

## 12.3 SUMMARY

Acrylonitrile has been shown to affect some terrestrial and aquatic plants at exposure concentrations of 9 to 100 mg/ℓ. Acrylonitrile is toxic to aquatic animals at exposure concentrations in the low milligrams per liter range. The reported acute $LC_{50}$ values for fish ranged between 10.1 and 70 mg/ℓ. Subchronic exposure of fish for 30 to 100 days resulted in $LC_{50}$ values of ≈2 mg/ℓ, with no evidence that a threshold concentration had been reached. Although the only tested invertebrate, *Daphnia magna*, has the lowest acute $LC_{50}$ value (7.6 mg/ℓ), this species was not adversely affected by chronic exposure to 3.6 mg/ℓ throughout its whole life cycle.

# 13. BIOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS

## 13.1 PHARMACOKINETICS

### 13.1.1 Absorption and Distribution

Young *et al*. (1977) studied extensively the pharmacokinetic and metabolic fate of acrylonitrile in male Sprague-Dawley rats by giving $^{14}C$-acrylonitrile with different routes and dose levels. When acrylonitrile was orally administered to rats, essentially all (95%) of the dose was absorbed. After 72 hours, the percentage (± the standard deviation) of recovered radioactivity accounted for 82 ± 9.64% and 104 ± 14.4% of the administered dose at 0.1 and 10 mg/kg, respectively (Table 13-1). The present recovery of administered radioactivity in the urine was much higher in the animals administered the higher dose, while both doses resulted in a 5% recovery of radioactivity in the feces.

The absorption of acrylonitrile through inhalation was also determined by Young *et al*. (1977). Animals were exposed to acrylonitrile vapor in a "nose only" chamber at concentrations of 5 and 100 ppm (11 and 217 $mg/m^3$) acrylonitrile. Following exposure at the higher dose, a significantly higher recovery was found in urine, but a smaller percentage was recovered in the expired air as $CO_2$ and in the body (Table 13-2).

Rogaczewska (1975) studied dermal absorption of acrylonitrile vapor in rabbits and found that the penetration rate of acrylonitrile vapor through the skin is about 1% in relation to the quantity absorbed through the lungs.

The plasma concentration of acrylonitrile (µg Eq/ml plasma) as a function of time, routes of administration (*per os* and intravenous), and dose levels (0.1, 1, and 30 mg/kg) has been studied (Young *et al*., 1977). Following oral and intravenous administration of $^{14}C$-acrylonitrile, typical plasma concentration versus time curves was observed. The biphasic disappearance of radioactivity

Table 13-1. Recovery of Radioactivity from Rats Given Single Oral Doses of 0.1 or 10 mg/kg $^{14}$C-Acrylonitrile[a] (Young *et al.*, 1977)

| | Percentage of Dose, Mean ± S.D. | |
|---|---|---|
| | 0.1 mg/kg | 10 mg/kg |
| Radioactivity in Urine | 34.22 ± 6.26[b] | 66.68 ± 10.60[b] |
| Radioactivity in Feces | 5.36 ± 1.43 | 5.22 ± 1.17 |
| Expired Air | | |
| Organics in carbon[c] | 0.09 ± 0.09 | 0.11 ± 0.06 |
| Organics in solvent[d] | 0.19 ± 0.19 | 0.20 ± 0.16 |
| $H^{14}CN$[e] | 0.07 ± 0.05 | 0.08 ± 0.03 |
| $^{14}CO_2$[f] | 4.56 ± 1.82 | 3.93 ± 1.79 |
| Body | 37.02 ± 6.09[b] | 26.61 ± 5.91[b] |
| Carcass | 24.24 ± 5.02[b] | 16.04 ± 1.87[b] |
| Skin | 12.78 ± 1.17 | 10.57 ± 4.55 |
| Cage Wash[g] | 0.86 ± 0.37 | 1.22 ± 0.41 |
| Total Recovery[h] | 82.37 ± 9.64[b] | 104.04 ± 14.40[b] |

[a] Two groups of rats housed individually in glass metabolism cages were given 0.1 mg/kg (4 rats) or 10 mg/kg (5 rats); excreta were collected at 8 hour intervals for 72 hours.

[b] The mean values for the two dose levels are different at the P=0.05 level of significance.

[c] Pittsburg activated coconut charcoal, 12 x 30 mesh.

[d] trap = 2-methoxyethanol

[e] trap = 0.02 M $Ag_2SO_4$ in 0.1 N $H_2SO_4$

[f] trap = 5 M ethanolamine in 2-methoxyethanol

[g] water-acetone

[h] The mean and S.D. of the recovery were calculated from the total recovery of the dose in individual rats.

Table 13-2. Recovery of Radioactivity from Rats Exposed by Inhalation to 5 or 100 ppm $^{14}$C-Acrylonitrile for 6 hours[a] (Young *et al.*, 1977)

| | Percentage of Recovered Dose[b] | |
|---|---|---|
| | 5 ppm | 100 ppm |
| Urine | 68.50 ± 9.38[c] | 82.17 ± 4.21[c] |
| Feces | 3.94 ± 0.97 | 3.15 ± 0.82 |
| $^{14}CO_2$ | 6.07 ± 1.58[c] | 2.60 ± 0.83[c] |
| Body | 18.53 ± 4.68[c] | 11.24 ± 2.85[c] |
| Cage Wash | 2.95 ± 3.95 | 0.85 ± 0.58 |

[a] Rats were exposed in a "nose only" chamber under dynamic air flow conditions for 6 hours. After exposure, rats were housed in glass metabolism cages and excreta were collected for 220 hours.

[b] Values are the mean ± S.D. for 4 rats per exposure level.

[c] The mean values for the two exposure levels are different at the P=0.05 level of significance.

indicated a pharmacokinetic two-compartment open model for elimination. The half-life values of alpha and beta phases, calculated by linear regression analysis, ranged from 3.5 to 5.8 hours and 50 to 77 hours, respectively.

Freshour et al. (1980) investigated the pharmacokinetic profile of intact acrylonitrile after intravenous or per os administration of acrylonitrile to male Fischer F344 rats. The plasma concentration of acrylonitrile versus time obtained after intravenous administration was characteristic of a one-compartment model with first-order elimination, but a biphasic elimination was observed following a 30 mg/kg oral administration. The half-life of first-order elimination ranged from 7.8 to 13.9 minutes after 30 mg/kg intravenous and per os doses, and the half-life for terminal phase after 30 mg/kg oral administration was 85 to 120 minutes.

Hashimoto and Kanai (1965) have studied the blood concentrations of acrylonitrile and cyanide as a function of time in relation to toxicity. When acrylonitrile was given to rabbits intravenously at a sublethal dose (30 mg/kg, LD50 = 50 mg/kg), acrylonitrile was biphasically eliminated, with 1 µg/ml of acrylonitrile remaining four hours after dosing. The cyanide concentration rose to about 1.5 µg/ml at 1.5 hours after dosing, and then gradually returned to zero at four hours. After injection of a lethal dose of acrylonitrile (75 mg/kg), the hydrogen cyanide concentration rose steadily until the death of the animal.

Tissue distribution of radioactivity (acrylonitrile and its metabolites) was determined in rats given a single oral or intravenous dose of $^{14}$C-acrylonitrile at doses of 0.1 and 10 mg/kg (Young et al., 1977). Acrylonitrile and its metabolites were distributed to all tissues examined (lung, kidney, liver, stomach, skin, blood, etc.); notably, high levels of radioactivity were observed in stomach, skin, and red blood cells regardless of route and dose level (Table 13-3). The high accumulation of radioactivity in the stomach wall following

Table 13-3. Distribution of Radioactivity in Selected Tissues of Rats Given $^{14}$C-Acrylonitrile (Young *et al.*, 1977)

| | Tissue to Plasma Ratios of $^{14}$C-Activity | | | |
|---|---|---|---|---|
| | i.v.[a] | | p.o.[b] | |
| | 2 hours<br>1 mg/kg | 24 hours<br>1 mg/kg | 72 hours<br>0.1 mg/kg | 72 hours<br>10 mg/kg |
| Lungs | 0.96 | 1.07 | 0.87 | 0.96 |
| Liver | 0.68 | 1.20 | 0.57 | 0.84 |
| Kidneys | 1.33 | 3.15 | 0.83 | 1.08 |
| Stomach Wall and Contents | 8.11 | 7.36 | 14.28 | 11.26 |
| Intestines | 0.72 | 0.31 | 1.06 | 0.99 |
| Duodenum | 0.85 | 0.68 | –[c] | – |
| Jejunum | 0.92 | 0.57 | – | – |
| Ileum | 1.01 | 0.44 | – | – |
| Cecum | 0.37 | 0.07 | – | – |
| Colon | 0.51 | 0.07 | – | – |
| Skeletal Muscle | – | 0.50 | 0.35 | 0.41 |
| Heart | 0.64 | 0.78 | – | – |
| Spleen | 0.98 | – | – | – |
| Brain | 0.65 | 0.76 | – | – |
| Thymus | 0.74 | – | – | – |
| Testes | 0.67 | – | – | – |
| Skin | 1.91 | 2.85 | – | – |
| Carcass | 0.54 | 0.73 | 2.10 | 2.70 |
| Packed Blood Cells | 3.33 | 7.16 | 0.71 | 0.55 |
| Plasma Concentration (μg Eq/ml) | 0.727 | 0.220 | 2.26[d] | 5.73[e] |
| | | | 0.022 | 1.437 |

[a] One rat per time. (i.v. = intravenous.)

[b] Average of 2 rats per dose. (p.o. = *per os*.)

[c] A dash indicates no analysis was performed.

[d] For all 4 rats in this group, the ratio was 2.18 ± 0.20 (Mean ± S.D.)

[e] For all 5 rats in this group, the ratio was 5.16 ± 1.19 (Mean ± S.D.)

intravenous acrylonitrile administration indicated that the high concentration in the stomach wall following acrylonitrile oral dosing was not due to poor absorption. Analysis of the stomach following intravenous administration showed that the radioactivity had increased from 30.33 µg Eq at 5 minutes to 68.64 µg Eq at 24 hours. The particular retention of acrylonitrile and its metabolites in the stomach seems in part due to enterogastric circulation (Young *et al*., 1977). The accumulation of radioactivity in blood was mainly due to covalent binding of acrylonitrile to macromolecules and lipids in the red blood cells (Ahmed and Patel, 1979; Ahmed *et al*., 1982). In other tissues, the amount of radioactivity declined rapidly with time because of excretion (Young *et al*., 1977; Ahmed and Patel, 1979; Ahmed *et al*., 1982).

Similar distribution patterns were obtained by Sandberg and Slanina (1980) using the technique of whole-body autoradiography following administration of labeled acrylonitrile to rats and monkeys. Male and female rats received $^{14}C$-acrylonitrile by intravenous injection (13 mg/kg) and were killed 1 minute, 20 minutes, 1 hour, 4 hours, and 7 days after treatment. Two monkeys were given 4 or 6 mg/kg acrylonitrile orally and killed 1 and 6 hours later, respectively. There was no difference in the distribution patterns of acrylonitrile between the two routes of administration in rats, except for the slower absorption time following oral dosing. High levels of activity were present in the blood and excretory pathways (bile, intestinal contents, and urine) with the liver, kidney, lung, and adrenal cortex also accumulating appreciable label. The stomach and hair follicles showed constant uptake of label throughout this study. Radioactivity was still present in animals killed seven days after administration of acrylonitrile. In fetuses exposed *in utero*, only the eye lens accumulated label at a higher concentration than that observed in maternal blood. The labeling pattern in monkeys was similar to that in rats except for a more pronounced activity in the liver.

### 13.1.2 Metabolism

The metabolism of acrylonitrile has been studied by many researchers. Dudley and Neal (1942) and Brieger et al. (1952) suggested that acrylonitrile is metabolized to cyanide, which is transformed to thiocyanate and eliminated in urine; however, less than one third of administered dose has been accounted for by this metabolic route (Gut et al., 1975). Acrylonitrile reacts with sulfhydryl groups through cyanoethylation, which prevents further metabolism to cyanide and thiocyanate (Hashimoto and Kanai, 1965). Other metabolic pathways have been suggested including formation of mercapturic acid (U.S. EPA, 1979), oxidation to $CO_2$ via cyanate ion (Boxer and Richards, 1952), and conjugation with D-glucuronic acid (Hoffman et al., 1976). Evidence from Gut et al. (1975), Wright (1977), and Young et al. (1977) suggested that the metabolism of acrylonitrile in mammals was related to dose level, route of administration, and species.

#### 13.1.2.1 Metabolism to Cyanide and Thiocyanate

Acrylonitrile is metabolized in laboratory animals to cyanide ($CN^-$), and then converted to thiocyanate ($SCN^-$). Evidence of this pathway has been presented by Dahm (1977). The excreted urine collected from orally-dosed $^{14}C$-acrylonitrile rats was analyzed by high pressure liquid chromatography (HPLC). The suspected thiocyanate peak matched the retention time of a thiocyanate standard. Another experiment by Dahm (1977) showed direct evidence that thiocyanate comes from the cyano group carbon of acrylonitrile. When rats were orally administered $^{14}C$-acrylonitrile, labeled on either the olefin or cyano group, the thiocyanate peak by HPLC was observed only when the cyano group was labeled. Ahmed and Abreu (1982) have shown that acrylonitrile is converted to cyanide in the brain and liver of rats by enzymes associated with the microsomal fraction.

Urinary excretion of thiocyanate following acrylonitrile administration at different dose levels and by various routes accounted for from 2 to 33% of dose (Gut et al., 1975; Hashimoto and Kanai, 1965). Gut et al. (1975) emphasized that the extent of conversion of acrylonitrile to cyanide and thiocyanate is both route- and species-dependent.

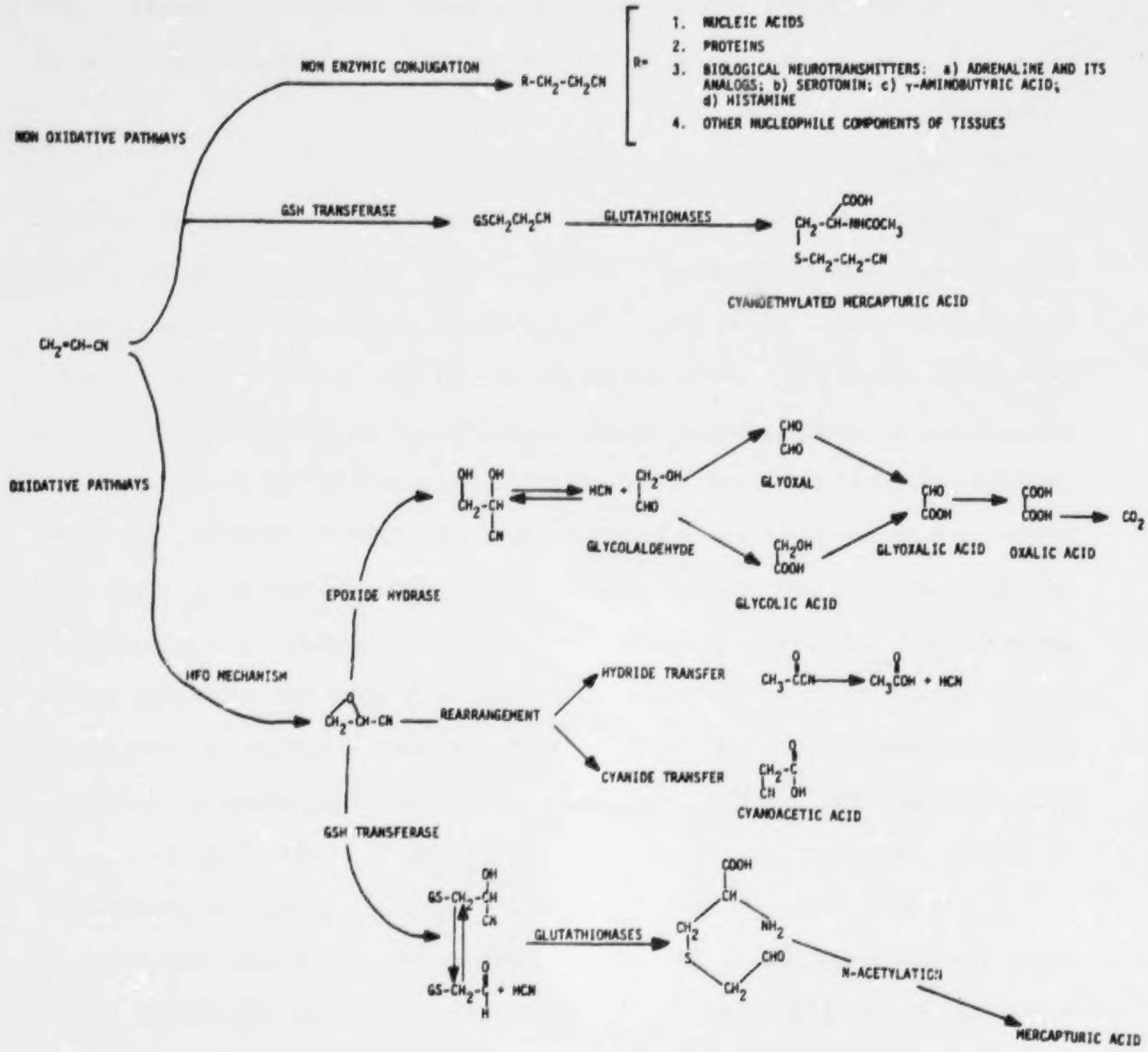
Using female Wistar rats, albino mice, and Chinese hamsters, Gut et al. (1975) studied the extent of the conversion of acrylonitrile to thiocyanate. In rats, there was higher thiocyanate excretion after oral administration (14.6 to 33.1%) than after intraperitoneal (2.2 to 5.7%), subcutaneous (4.6%), or intravenous (1.2%) injection. After oral dosing, thiocyanate excretion showed a distinct lag period (4 hours), which suggested that acrylonitrile was not appreciably metabolized shortly after oral dosing (Gut et al., 1975). In mice, there was also a higher percentage of thiocyanate excreted after oral (35%) than after intraperitoneal (8 to 10%) and intravenous (11%) injections. The portion of acrylonitrile converted to thiocyanate was approximately the same for mice and for rats. The higher total recovery of thiocyanate in mice compared to rats indicated that mice may have a higher metabolic capacity and a lower binding capacity for acrylonitrile (Gut et al., 1975). Hamsters were similar to rats and mice in that more thiocyanate was excreted in urine after oral than after intraperitoneal administration of acrylonitrile. In contrast to rats, elimination of thiocyanate in mice and hamsters after oral dosing showed no lag period. Gut et al. (1975) indicated that body size may be an influencing factor.

Pretreatment of rats with phenobarbital (a microsomal inducer), SKF525-A (a microsomal inhibitor), cysteine, or dimercaprol did not significantly influence the excretion of thiocyanate in the urine after the administration of acrylonitrile. Simultaneous intraperitoneal administration of acrylonitrile and thiosulfate to rats and mice increased thiocyanate elimination twofold in rats and threefold in mice, but no effect of thiosulfate was observed after oral administration of acrylonitrile (Gut *et al*., 1975). The lack of effect of microsomal enzyme induction or inhibition on acrylonitrile-thiocyanate balance indicated that the route-dependent differences in thiocyanate elimination observed within one strain of animals are caused by factors affecting the tissue distribution of acrylonitrile (Gut *et al*., 1975).

### 13.1.2.2 Reaction with Sulfhydryl Groups

Acrylonitrile reacts with sulfhydryl compounds in laboratory animals through cyanoethylation (Hashimoto and Kanai, 1965; Gut *et al*., 1975; Dahm, 1977 and Langvardt *et al*., 1979); the reaction products are not further metabolized by animals but rather are excreted unchanged (Gut *et al*., 1975). It has also been demonstrated in hamsters following intraperitoneal injection, that acrylonitrile treatment resulted in a rapid decrease (within 4 hours) in the levels of glutathione in both the liver and brain (Zitting et al., 1981). Hashimoto and Kanai (1965) showed that acrylonitrile rapidly forms stable conjugates *in vitro* with materials that have active hydrogen atoms, such as L-cysteine and L-glutathione. That acrylonitrile forms conjugates with cysteine *in vivo* was also demonstrated and the chemical structures of the metabolites were identified as S-(2-cyanoethyl)cysteine (Gut *et al*., 1975; Dahm, 1977) and N-acetyl-S-(2-cyanoethyl) cysteine(cyanoethylated mercapturic acid) (Dahm, 1977; Langvardt *et al*., 1979).

It has been suggested that acrylonitrile reacts *in vitro* with glutathione (GSH) via GSH transferase, but the conjugation was determined indirectly by measuring the disappearance of GSH substrates (Boyland and Chasseaud, 1967). Recently, the presence of cyanoethylated mercapturic acid in rat urine was confirmed (U.S. EPA, 1979). A proposed scheme for the various metabolic pathways of acrylonitrile biotransformation is presented in Figure 13-1.



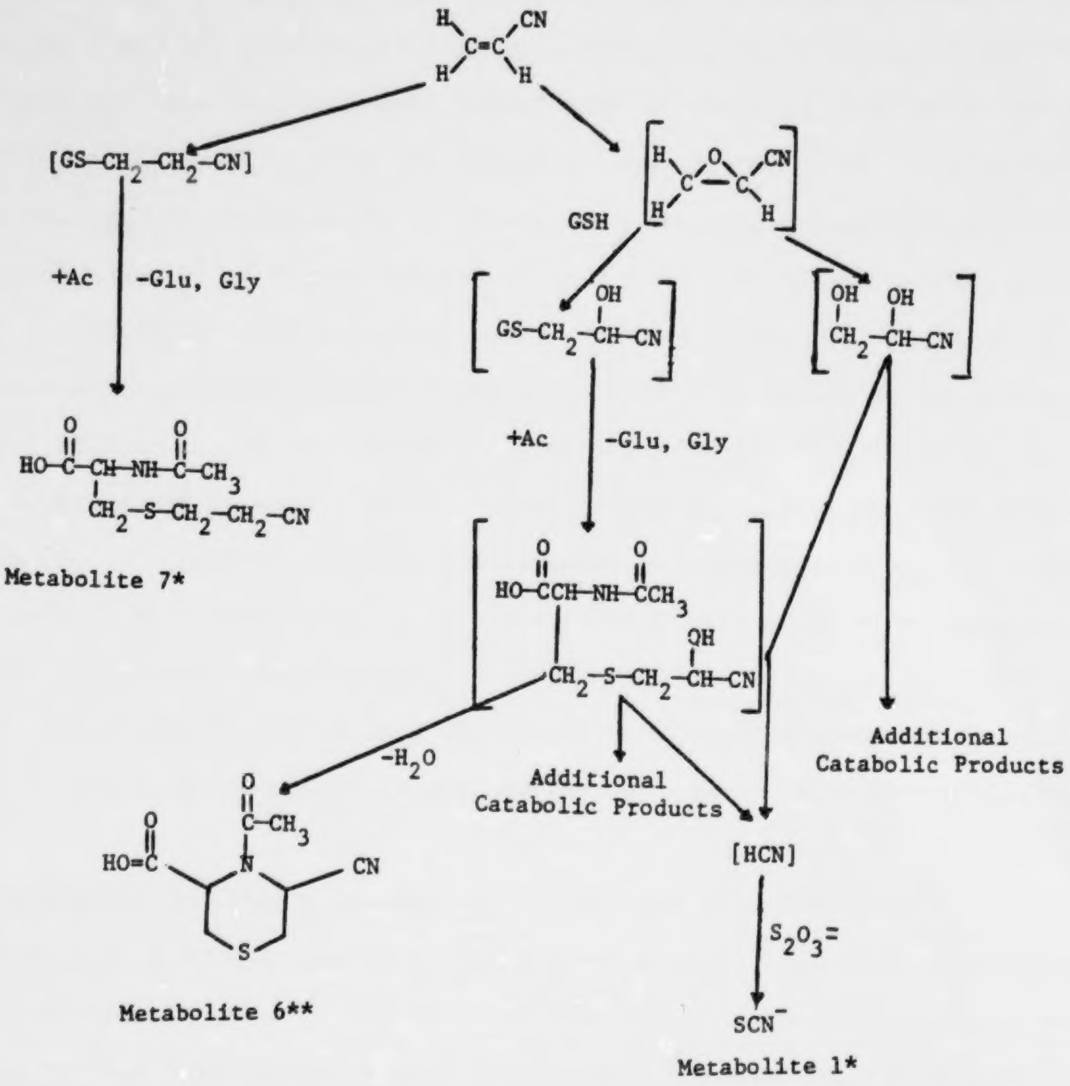
NON OXIDATIVE PATHWAYS
NON ENZYMIC CONJUGATION
R-CH2-CH2CN
R=
1. NUCLEIC ACIDS
2. PROTEINS
3. BIOLOGICAL NEUROTRANSMITTERS: a) ADRENALINE AND ITS ANALOGS; b) SEROTONIN; c) γ-AMINOBUTYRIC ACID; d) HISTAMINE
4. OTHER NUCLEOPHILE COMPONENTS OF TISSUES
GSH TRANSFERASE
GSCH2CH2CN
GLUTATHIONASES
COOH
CH2-CH-NHCOCH3
S-CH2-CH2-CN
CYANOETHYLATED MERCAPTURIC ACID
CH2=CH-CN
OXIDATIVE PATHWAYS
OH OH
CH2-CH
CN
HCN +
CH2-OH
CHO
GLYCOLALDEHYDE
CHO
CHO
GLYOXAL
CH2OH
COOH
GLYCOLIC ACID
CHO
COOH
GLYOXALIC ACID
COOH
COOH
OXALIC ACID
CO2
EPOXIDE HYDRASE
MFO MECHANISM
O
CH2-CH-CN
REARRANGEMENT
HYDRIDE TRANSFER
O
CH3-CCN
O
CH3COH + HCN
CYANIDE TRANSFER
O
CH2-C
CN OH
CYANOACETIC ACID
GSH TRANSFERASE
OH
GS-CH2-CH
CN
O
GS-CH2-C + HCN
H
GLUTATHIONASES
COOH
CH
CH2
S
NH2
CHO
CH2
N-ACETYLATION
MERCAPTURIC ACID

Figure 13-1. Proposed Pathways for Acrylonitrile Biotransformation (U.S. EPA, [1979])

Langvardt _et_ _al_. (1979) detected 7 urinary metabolites of acrylonitrile, by high pressure liquid chromatography, in rats following administration of acrylonitrile (30 mg/kg) by oral intubation. Of the 7 metabolites, the 3 major metabolites (metabolites 1, 6, and 7) were further investigated to identify chemical structures. Metabolite 7 was confirmed to be N-acetyl-S-(2-cyanoethyl) cysteine, and metabolite 1 was thiocyanate, as indicated by _in_ _vivo_ labeling patterns when animals were given 2,3-$^{14}$C acrylonitrile or 1-$^{14}$C acrylonitrile, and retention times and fragmentation patterns on GC-MS. Metabolite 6 was tentatively identified as N-acetyl-3-carboxy-5-cyanotetrahydro-1,4-2H-thiazine since it had an apparent molecular weight 2 units less than metabolite 7 and formed artifacts similar to metabolite 7 on being reduced to dryness under $N_2$ gas. No authentic standard to metabolite 6 could be synthesized. By using analogues to known metabolic pathways for other halogenated vinyl compounds, Langvardt _et_ _al_. proposed a possible metabolic pathway of acrylonitrile which proceeds through the formation of an epoxide intermediate (Figure 13-2). The authors suggest that the epoxide metabolite may be an important factor in the toxicity of acrylonitrile.

Abreu and Ahmed (1980) also demonstrated that an _in_ _vitro_ system, employing a rat liver microsomal fraction and NADPH, was capable of forming cyanide from acrylonitrile. Using microsomal fractions from rats induced with phenobarbital or Aroclor 1254 increased the production of cyanide, while addition of SKF525-A to the reaction mixture inhibited the formation of cyanide. These results were consistent with a metabolic pathway employing an epoxide as an intermediate.

In a similar study using [1-$^{14}$C]- or [2,3-$^{14}$C]-acrylonitrile incubated with rat liver microsomes, Guengerich _et_ _al_. (1981) isolated labeled compounds which co-chromatographed on high performance liquid chromatography (HPLC) with authentic 2-cyanoethylene oxide. The formation of the epoxide was enhanced by the inhibition of epoxide hydrolase. Addition of epoxide hydrolase to a solution of


[GS–CH2–CH2–CN]
+Ac
-Glu, Gly
GSH
-H2O
Additional Catabolic Products
Additional Catabolic Products
[HCN]
S2O3=
SCN-
Metabolite 7*
Metabolite 6**
Metabolite 1*

*Confirmed Assignment
**Tentative Assignment
[ ]Hypothetical Assignment

Figure 13-2. Proposed Scheme for Metabolism of Acrylonitrile by the Rat (Langvardt et al., 1980)

0.5 mM 2-cyanoethylene oxide resulted in the destruction of the epoxide at a rate of 1.7 mmol/min, and the formation of HCN. Both acrylonitrile and 2-cyanoethylene oxide when mixed with GSH (reduced glutathione) reacted non-enzymatically, and HCN was released in the reaction with the epoxide. The epoxide reacted less rapidly than did acrylonitrile in this system, however, when a cytosol preparation from rat liver was used, the rate of GSH conjugate formation was 309 and 31 mmol/mg/min, respectively, for 2-cyanoethylene oxide and acrylonitrile. The cytosol from rat brain and human liver was inactive in forming GSH conjugates with acrylonitrile. These preparations were active with 2-cyanoethylene oxide, although the activity was diminished by more than an order of magnitude when compared to that in the rat liver preparation. A metabolite with a mass spectrum similar to that which Langvardt et al. (1980) ascribed to 4-acetyl-3-carboxy-5-cyanotetrahydro-1,4-2H-thiazine was obtained by non-enzymatic reaction of 2-cyanoethylene oxide with N-acetylcysteine. This evidence supports a metabolic pathway in which acrylonitrile is metabolized by microsomal enzymes through an epoxide intermediate to HCN. It was demonstrated that 2-cyanoethylene oxide non-enzymatically bound to both protein and DNA, and that acrylonitrile did not, indicating that the 2-cyanoethylene oxide may be an important metabolite in the toxicity and carcinogenicity of acrylonitrile (Langvardt et al., 1980).

Wright (1977) studied the dose-related and species strain-related metabolism of acrylonitrile. Spartan rats, a Charles River rat, and rhesus monkeys were orally dosed with cyano-labeled acrylonitrile and the urinary metabolites were determined (Table 13-4). In rats, a lower dose (0.1 mg/kg) of acrylonitrile resulted in about 90% of the administered dose being metabolized to cysteine conjugates. At a higher dose (30 mg/kg), a great portion of administered acrylonitrile was excreted as thiocyanate and unidentified metabolite "C." Wright

Table 13-4. Urinary Metabolites Following the Oral Administration of C-1 (Cyano) Labeled Acrylonitrile (Wright, 1977)

| Animal | Number | Dose (mg/kg) | Percent of Radioactivity in Urinary Metabolites[a] | | |
|---|---|---|---|---|---|
| | | | Cysteine-Conjugates | "C" (unidentified) | Thio-cyanate |
| Spartan Rat | 1 | 0.1 | 88.6 | 3.5 | 8.0 |
| Spartan Rat | 1 | 30.0 | 37.3 | 20.5 | 42.1 |
| Charles River Rat | 1 | 30.0 | 47.5 | 13.8 | 38.8 |
| Rhesus Monkey | 1 | 0.1 | 91. | 6. | 3. |
| Rhesus Monkey | 1 | 30.0 | 84. | 14. | 2. |

[a] 0-20 hours in rats and 0-24 hours in monkeys.

(1977) suggested that conjugation with cysteine is the preferred metabolic pathway for acrylonitrile but has limited capacity. No significant difference between the two strains of rats given a 30 mg/kg oral dose of acrylonitrile was observed. As shown in Table 13-4, there is no significant difference in the proportion of metabolites excreted in urine by rhesus monkeys following 0.1 and 30 mg/kg of acrylonitrile. This may indicate that the rhesus monkey has a greater metabolic capacity in conversion of acrylonitrile to cysteine-conjugates than does the rat.

#### 13.1.2.3 Minor Metabolites

In addition to the cyanide and thiocyanate formed by acrylonitrile's metabolism, acrylonitrile-glucuronide was formed and excreted in rat urine. Hoffman _et al_. (1976) suggested that acrylonitrile is initially hydrolyzed, and then undergoes a condensation reaction with UDP-glucuronic acid.

Young _et al_. (1977) identified $CO_2$ as a metabolite of acrylonitrile. Carbon dioxide was expired in the breath and accounted for 5 to 6% of the administered dose of acrylonitrile. Boxer and Richards (1952) demonstrated that cyanide and thiocyanate are in a dynamic equilibrium in the rats and that, to a large extent, cyanide and thiocyanate carbon are oxidized directly to $CO_2$. Thus, $CO_2$ may arise as a product of cyanate metabolism.

The majority of the administered dose of acrylonitrile was metabolized and excreted; however, Hashimoto and Kanai (1965) and Hoffmann _et al_. (1976) estimated that 15% of an administered acrylonitrile dose is eliminated unchanged in the urine and breath.

#### 13.1.2.4 Route and Dose Dependence of Acrylonitrile Metabolism

The metabolic fate of acrylonitrile and its dependence on dose level and route of administration have been studied by many researchers (Gut _et al_., 1975; Wright, 1977; Young _et al_., 1977). Gut _et al_. (1975) indicated that the formation of both cyanide and thiocyanate are dose- and route-related. Wright (1977) found that the reaction of acrylonitrile with sulfhydryl groups is dose-dependent. Recently, Young et al. (1977) quantitatively isolated three metabolites designated as "A," "C," and "E" as well as $CO_2$ in male Sprague-Dawley rats given doses of $^{14}C$-acrylonitrile by several routes. Metabolites A, C, and E, which accounted for more than 95% of the total radioactivity, were excreted primarily in the urine, while $CO_2$ was excreted in the breath. The chemical structures of metabolites A, C, and E were cited by Keresztesy et al. (1977) as N-acetylated conjugates of cysteine, thiocyanate, and thiocyanate, respectively (apparently there was an error in the paper; thus, the reader should not assume that the structures of C and E are the same), but there was no evidence for structure identification in the original report (Young et al., 1977).

The proportion of the metabolites A, C, and E in various rat fluids was route-and dose-dependent (see Table 13-5). The 0-72 hour urine sample showed the highest amount of metabolite C (73%) after an oral dose of 0.1 mg/kg, but the maximum amount of metabolite A (61%) was observed after a higher oral dose of 10 mg/kg (Young et al., 1977).

When rats were exposed to acrylonitrile vapor at a concentration of 5 ppm (11 mg/m$^3$) for 6 hours, 61% of metabolite E was excreted in the urine, while a dose of 100 ppm (217 mg/m$^3$) resulted in equal proportions of the three metabolites.

The predominant metabolite excreted in bile after a 1 mg/kg intravenous dose was metabolite C (91%). For stomach, red blood cell, and plasma, metabolite E accounted for 93, 83, and 42% of total radioactivity, respectively.

### 13.1.3 Summary and Conclusions

Acrylonitrile is readily absorbed in animals following ingestion or inhalation, while dermal absorption is poor and occurs at ≈1% of that of the lungs.

**Table 13-5. Metabolites of $^{14}$C-Acrylonitrile Separated from Various Fluids of Rats by High Pressure Liquid Chromatography (HPLC) (Young et al., 1977)**

| Sample | Percentage of Total $^{14}C^{a}$ | | |
|---|---|---|---|
| | A | C | E |
| Urine, 0-72 hr. | | | |
| 0.1 mg/kg, p.o. | 12 | 73 | 15 |
| 10.0 mg/kg, p.o. | 61 | 8 | 32 |
| 5 ppm | 9 | 30 | 61 |
| 100 ppm | 32 | 33 | 35 |
| Bile[b], 1 hr. | 2 | 91 | 1 |
| *In vitro*[c] | 17 | 78 | 1 |
| Stomach[b,d], 24 hr. | 4 | 3 | 93 |
| RBC[b,d], 24 hr. | 7 | 10 | 83 |
| Plasma[b], 24 hr. | 28 | 28 | 42 |

[a] Metabolites A, C, and E were the major metabolites separated by HPLC. Values are expressed as percentages of the total radioactivity in each sample applied to the column. The total percentages of A, C, and E are less than 100% when minor metabolites B and D are present.

[b] A dose of 1 mg $^{14}$C-acrylonitrile/kg was given intravenously.

[c] Metabolites extracted from reaction mixtures with ethanol. The source of enzyme was rat liver 9000 x g supernatant fluid.

[d] Ethanol extracts of stomach or red blood cells (RBC). About 80% of the radioactivity was extracted.

Following absorption of radiolabeled acrylonitrile, the radioactivity disappears in a biphasic manner, with a half-life for the first phase of 3.5 to 3.8 hours and the second phase of 50 to 77 hours (Young et al., 1977). The predominant route of elimination is through the urine. The routes of elimination are dose-related with the percent eliminated through the urine less for small doses as compared to larger doses, while the relative amount retained by the carcass is greater for the small dose as compared to a larger dose. Acrylonitrile is metabolized to cyanide, which is transformed to thiocyanate and by cyanoethylation of sulfhydryl groups to S-(2-cyanoethyl)cysteine, followed by elimination of these metabolites in the urine. Other minor metabolites are formed from acrylonitrile. The toxicity of acrylonitrile is caused by both the acrylonitrile molecule itself and its metabolites.

## 13.2 ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

### 13.2.1 Acute Toxicity

The initial investigation of the acute toxic effects of acrylonitrile began in the 1940s when industrial use of acrylonitrile increased dramatically due to the production of oil-resistant synthetic rubber. These studies included both laboratory investigations in animals and compilations of case histories of human intoxication that had occurred in the workplace. Since these early cases of intoxication, occupational exposure to acrylonitrile has been greatly limited by engineering controls, and no recent cases of acute human intoxication have occurred.

#### 13.2.1.1 Acute Systemic Human Toxicity

Non-fatal intoxication by acrylonitrile has been reported in workers who clean polymerizers in plants that manufacture synthetic rubber (Wilson, 1944; Wilson _et al_., 1948). During these operations, workers were exposed to between 16 and 100 ppm of acrylonitrile for periods of 20 to 45 minutes. All workers complained of nasal irritation and an oppressive feeling in the upper respiratory passages. Dull headaches, nausea, and subjective feelings of apprehension and nervous irritability were also common complaints following mild intoxication. In more severe cases of intoxication, a low grade anemia and mild jaundice occurred. All exposed workers appeared to recover fully, with the exception of one who had severe jaundice. This worker complained of lassitude and fatigue a year later, although no pathological signs could be found. Similar symptoms of headache, vertigo, nausea, and vomiting were reported by a chemist engaged in distilling acrylonitrile (Sartorelli, 1966). Tremors, uncoordinated movement, and convulsions were also experienced. As with the other cases of intoxication, complete recovery occurred. Baxter (1979) has recently summarized the symptoms of acrylonitrile poisoning in man, in order of occurrence, as follows: irritation of eyes and nose, limb weakness, labored breathing, dizziness and impaired judgement, cyanosis and nausea, collapse, irregular breathing, and convulsions, possibly followed by cardiac arrest. A few additional case histories of occupational exposure to acrylonitrile were presented in an IARC Monograph (1979).

Five cases of acute lethal human intoxication have been reported (Davis et al., 1973) following fumigation of domiciles with acrylonitrile mixed with either 66% carbon tetrachloride or 70% methylene chloride (v/v). It is not clear whether acrylonitrile or the other component of the fumigant was the causative agent in these deaths; however, one patient had measurable levels (0.05 mg%) of cyanide in his blood at autopsy, and, in another case, the odor of cyanide was noted in the tissue. The presence of cyanide only indicates that acrylonitrile was metabolized prior to death. The only consistent symptom in these victims was nausea as an initial indication of intoxication. On autopsy, no gross abnormalities that could be attributed to the fumigant were noted.

In a report of four case histories of human disease following fumigation of domiciles with acrylonitrile and carbon tetrachloride mixtures, Radimer et al. (1974) described the appearance of toxic epidermal necrolysis. The first symptoms appeared between 11 and 21 days following rehabitation of the house (all houses were declared safe by the exterminators). Symptoms observed in victims on entering the hospital resembled those of extensive second-degree burns. Three of the patients died 2 to 9 days after hospitalization and one recovered and was discharged in 10 days. Biopsy samples showed blisters at the junction of the dermis with a necrotic epidermis. It was believed that exposure occurred through inhalation rather than skin absorption. Although a participating role for carbon tetrachloride could not be overruled, the lack of reports of epidermal disease following carbon tetrachloride exposure made the authors suspect that acrylonitrile was the toxic agent.

### 13.2.1.2 Acute Systemic Animal Toxicity

Acrylonitrile is a highly toxic compound to laboratory animals, regardless of the route of administration. In rats, the oral LD50 of acrylonitrile was determined to be 113 mg/kg by Smyth et al. (1969) and the minimal fatal oral dose in rats was reported to be 150 mg/kg (Wilson et al., 1948). The LD50 in Wistar rats following subcutaneous or intraperitoneal injection of acrylonitrile was 80 mg/kg and 100 mg/kg, respectively (Knoblock et al., 1971). Benes and Cerna (1959) had reported the oral LD50 of acrylonitrile in both rats and mice as 78 mg/kg and 27 mg/kg, respectively. The approximate lethal doses in mice have been determined for acrylonitrile administered intragastrically and intraperitoneally, and were greater than 20 mg/kg and 15 mg/kg, respectively (McOmie, 1943). The symptoms of intoxication in rats were briefly described by Wilson et al. (1948) as respiratory changes, cyanosis, and convulsions followed by death. For the investigation of acute toxic effects of acrylonitrile, exposure by inhalation has been studied at length since this is the route of greatest concern in industrial exposure.

Dudley and Neal (1942) exposed Osborne-Mendel rats to atmospheres of 90 to 2445 ppm acrylonitrile for 30 minutes to 8 hours. The onset of symptoms and the lethality of acrylonitrile were strictly dependent on the length of exposure and the concentration of acrylonitrile (Table 13-6). Short periods of exposure [665 ppm (1443 mg/m$^3$) for 30 minutes] produced only mild effects, while a similar dose given over a long exposure period [635 ppm (1378 mg/m$^3$) for 4 hours] was fatal to all the animals.

Smyth and Carpenter (1948) observed fatalities in all 6 Sherman strain rats following a 4-hour exposure to 1000 ppm (2170 mg/m$^3$) acrylonitrile, and no deaths from a 500 ppm (1085 mg/m$^3$) exposure. In a second study, however, Carpenter et al. (1949) reported that 2 to 4 rats (Sherman strain) of a group of 6 animals succumbed following a 500 ppm (1085 mg/m$^3$) exposure for 4 hours. Brieger et al. (1952) exposed Wistar rats to 100 ppm (217 mg/m$^3$) acrylonitrile for 7 hours, with 4 of 20 animals dying. A similar dose [130 ppm (282 mg/m$^3$) for 4 hours] was a non-lethal dose in the study of Dudley and Neal (1942) (Table 13-6). Due to insufficient data, it is not possible to determine whether the observed differences in susceptibility of rats in these experiments were a result of mere experimental uncertainty, or were related to variations in strains of rats used, sex of the animals (this was not reported in any of the studies), or age of the animals (all animals were between 100 and 150 g, except the Osborne-Mendel rats, which were 295 g).

There was considerable species variation in the susceptibility of animals to the lethal effects of acrylonitrile, with the guinea pig being the most resistant species and the dog the most sensitive (Table 13-7). Many of the animals did not die during the exposure period but rather succumbed to the toxic

Table 13-6. Summary of Results of Exposures of Rats to Acrylonitrile[a] (adapted from Dudley and Neal, 1942)

| Length of Exposure (hours) | Concentration (ppm) | Dying During Exposure (%) | Total Deaths (%) | Severity of Effects and Remarks[b] |
|---|---|---|---|---|
| 1/2 | 2445 | 0 | 0 | Marked. Slight residual effects to 24 hrs. |
| | 1490 | 0 | 0 | Marked. No residual effects in 24 hrs. |
| | 1270 | 0 | 0 | Marked. No residual effects in 24 hrs. |
| | 665 | 0 | 0 | Moderate, transitory. |
| 1 | 2445 | 0 | 81 | Deaths in 4 hrs. Show some slight effects 24 hrs after exposure. |
| | 1490 | 0 | 25 | Deaths in 4 hrs. Show some slight effects 24 hrs after exposure. |
| | 1270 | 0 | 0 | Marked. Showed some effects 24 hrs after exposure. Normal in 48 hrs. |
| | 665 | 0 | 0 | Marked, transitory. |
| 2 | 1260 | 0 | 100 | Fatal. Dead in 4 hrs after exposure. |
| | 595 | 0 | 6 | Marked, transitory. |
| | 305 | 0 | 0 | Slight, transitory. |
| 4 | 635 | 50 | 100 | Fatal. Dead in 4 hrs after exposure |
| | 315 | 25 | 31 | Marked. Survivors show no effects in 24 hrs. |
| | 130 | 0 | 0 | Slight transitory effects. |
| 8 | 320 | 94 | 94 | Fatal |
| | 270 | 44 | 44 | Marked. Survivors show no effects in 24 hrs. |
| | 210 | 6 | 6 | Marked, transitory. |
| | 135 | 0 | 0 | Moderate, transitory. |
| | 90 | 0 | 0 | Slight discomfort only. |

[a] 16 rats were exposed at each concentration for length of time shown.

Rats used for these studies were adult albinos, pure Osborne-Mendel; average weight, 295 grams.

[b] Symptoms included respiratory distress and convulsions in cases of fatal exposure.

Table 13-7. Minimal Lethal Concentration of Acrylonitrile During Four-Hour Exposure

| Animal Species | PPM of Acrylonitrile | Fatalities/No. Challenged | Reference |
|---|---|---|---|
| Guinea pig | 575 | 10/16 | Dudley and Neal, 1942 |
| Cat | 600 | 16/16 | Dudley and Neal, 1942 |
| Rat | 315 | 5/16 | Dudley and Neal, 1942 |
| Mice[a] | 415 | 3/16 | McOmie, 1943 |
| Rabbit | 260 | 16/16 | Dudley and Neal, 1942 |
| Rhesus Monkey[a] | 75 | 1/3 | Brieger _et al_., 1952 |
| Dog[b] | 65 | 1/2 | Dudley and Neal, 1942 |

[a] Exposure time was three hours instead of four hours.

[b] The great sensitivity of dogs was confirmed in the study of Brieger _et al_., 1952.

effects later. The number of fatalities given in the table indicates the total number of animals that died during the exposure and observation period. The concentrations given in Table 13-7 should not be interpreted as the minimum lethal doses in the cases where a substantial number of challenged animals died; these were the lowest concentrations reported but are presumably higher than would be the minimum lethal doses.

The symptoms of intoxication were similar in all species except dogs and guinea pigs. As reported by Dudley and Neal (1942), the symptoms following lethal exposures included gasping (particularly of the abdominal type), spasm-like convulsive movements of the abdominal wall, general convulsions, and coma followed by death. With less severe exposure, hyperactivity and flushing (particularly in the Rhesus monkeys) were noted along with indication of slight irritation of the mucous membranes. Animals that were removed from the acrylonitrile atmosphere prior to the gasping stage recovered completely with no therapeutic attention (McOmie, 1943). Dogs showed all the same symptoms as other species upon exposure to a lethal concentration of acrylonitrile; however, for the dogs that did recover, the residual effects of poisoning lasted for a longer period of time (Dudley and Neal, 1942). The slow recovery included refusing to eat for 10 days following exposure in 1 animal, and, for another, 3 days of partial paralysis of the hind legs. In the most resistant species, the guinea pig, symptoms included watering of the eyes, nasal discharge, and coughing. When death did occur, it followed exposure by 3 to 6 days. Dudley and Neal (1942) concluded that death of guinea pigs was due to mucous membrane and pulmonary irritation and lung edema.

### 13.2.1.3 Mechanisms of Acute Toxicity

Early observation of animal experiments and human intoxication suggested that acrylonitrile toxicity was due to the liberation of cyanide following metabolism. Dudley et al. (1942) compiled data comparing the toxic levels of hydrocyanic acid and of acrylonitrile in a number of species of experimental animals (Table 13-8). Both in the quantities needed for intoxication and in the symptoms observed, acrylonitrile appeared to act in a manner similar to that of hydrocyanic acid. Furthermore, in humans, the gross signs and symptoms of acrylonitrile and cyanide poisoning were similar (Brieger et al., 1952).

With human exposure, the main biochemical evidence in support of the theory that acrylonitrile toxicity occurred through the action of cyanide was the presence of thiocyanate in the blood and urine of acrylonitrile-exposed individuals. Thiocyanate is the end product of the body's detoxification of cyanide. Following occupational exposure to acrylonitrile, maximum serum thiocyanate levels occurred immediately after exposure, while urinary maxima were reached between 24 and 48 hours (Mallette, 1943). The persistence of thiocyanate in the serum was proportional to the level of exposure, with blood levels normal at 2.5 hours after an exposure of 22 ppm (48 mg/m$^3$) acrylonitrile for 30 minutes and blood levels not returning to normal values by 12 hours after an exposure of 50 ppm (108 mg/m$^3$) for 30 minutes (Wilson et al., 1948). Due to the quantitative correlation between acrylonitrile exposure and urinary and blood thiocyanate concentrations, measurement of levels of thiocyanate was suggested as a method of monitoring acrylonitrile absorption in individuals (Mallette, 1943).

To determine whether acrylonitrile acts by the same mechanism as cyanide, animal models have been used to identify and compare cyanide metabolites in animals exposed to acrylonitrile. Cyanide acts by complexing with heavy metal ions, alone or as part of organic molecules, and the ultimate toxic effect is due to the complex of cyanide with the ferric ion in the cytochromes. This causes disruption in cytochrome function and cellular anoxia. During cyanide intoxication, two less toxic metabolites--cyanmethemoglobin and thiocyanate--are formed

Table 13-8. Comparison of the Effects of Acrylonitrile and of Hydrocyanic Acid on Various Species of Animals (Dudley and Neal, 1942)

| | Acrylonitrile | | | | Hydrocyanic Acid | | |
|---|---|---|---|---|---|---|---|
| | Concentration | | | | | | |
| | | Calculated as HCN | | | Concentration | | |
| Species | mg/1 | mg/1 | ppm | Symptoms | mg/1 | ppm | Symptoms |
| Rats | 0.28 | 0.14 | 130 | Slight transitory effect | 0.12 | 110 | Fatal in 1/2 hours |
| | 0.21 | 0.10 | 100 | Slight transitory effect | | | |
| Guinea pigs | 1.25 | 0.62 | 575 | Fatal during or after exposure | 0.35 | 315 | Fatal concentration |
| | 0.58 | 0.29 | 265 | Slight transitory effect | 0.23 | 200 | Tolerated for 1 1/2 hours without symptoms |
| Rabbits | 0.56 | 0.28 | 260 | Fatal during or after exposure | 0.35 | 315 | Fatal concentration |
| | 0.29 | 0.15 | 135 | Marked transitory effect | 0.13 | 120 | No marked toxic symptoms |
| | 0.21 | 0.10 | 100 | Slight transitory effect | | | |
| Cats | 0.60 | 0.30 | 275 | Markedly toxic | 0.35 | 315 | In 2 min respiratory paralysis, in 5 to 10 min. death |
| | 0.33 | 0.17 | 153 | Markedly toxic, sometimes fatal | 0.14 | 125 | Markedly toxic in 6 to 7 min |
| Dogs | 0.24 | 0.12 | 110 | Fatal to 3/4 of the dogs | 0.125 | 115 | Fatal to dogs |
| | 0.213 | 0.11 | 100 | Convulsions + coma, 0 death | 0.1 | 90 | May be tolerated for hours, death after exposu |
| | 0.12 | 0.06 | 56 | Transitory paralysis, 1 dog died | 0.07-0.04 | 63-35 | Vomiting, convulsions, recovery, may be fatal |
| | 0.063 | 0.03 | 30 | Very slight effects | 0.035 | 30 | May be tolerated |
| Monkeys | 0.33 | 0.17 | 153 | Definite toxic effects | 0.14 | 125 | Distinctly toxic after 12 min |

which competitively inhibit the toxic action of cyanide. The ability of cyanide antidotes, which facilitate the formation of competitive metabolites of the cyanide ion, to protect against acrylonitrile poisoning has also been investigated.

Cyanide has been found at various levels in animals after exposure to acrylonitrile. Dudley and Neal (1942) examined the liver, kidney, and gluteus muscle of rats and guinea pigs immediately after death due to inhalation of acrylonitrile and could find no detectable levels of cyanide. Hashimoto and Kanai (1965), however, detected cyanide at 2.5 to 3.5 µg/ml in blood of rabbits following intravenous administration of acrylonitrile (75 mg/kg) and in rats and guinea pigs after intraperitoneal administrations of 100 and 125 mg/kg, respectively. Cyanide was also detectable in the blood of both dogs and Rhesus monkeys following inhalation exposure to acrylonitrile, but not in the blood of rats (Table 13-9). In dogs, the levels of cyanide in the blood were proportional to the extent of exposure to acrylonitrile. This was the only study that found a positive correlation between the sensitivity of the species exposed to acrylonitrile (Table 13-7) and the level of cyanide in the blood.

Both cyanide metabolites, thiocyanate and cyanmethemoglobin, have been detected in the blood (and urine, in the case of thiocyanate) of animals after exposure to acrylonitrile. Brieger et al. (1952) compared the levels of thiocyanate in the blood of dogs with the levels in the blood of rats, a species which was less sensitive to the toxic effects of acrylonitrile (Table 13-9). An inverse relationship was demonstrated between the levels of thiocyanate, levels of cyanide, and degree of sensitivity, with the species less sensitive to acrylonitrile poisoning having lower blood cyanide and higher thiocyanate levels. The authors were surprised at the high thiocyanate levels as compared to the cyanide levels in the blood from monkeys. Cyanmethemoglobin was also detected in the

Table 13-9. Cyanide and Thiocyanate in Blood of Animals Exposed to Acrylonitrile (adapted from Brieger *et al.*, 1952)

| Species | Acrylonitrile in Air (ppm) | Length of Exposure | Cyanide in Blood (µmol/100 ml) | Thiocyanate mg/100 ml[a] | |
|---|---|---|---|---|---|
| | | | | Blood | Urine |
| Rats | 100 | 7 | 1 | 6.63[b] | 2.2 |
| Dogs | 100 | 1 | 2.93 | 0.45 | --- |
| Dogs | 100 | 7 | 10.10 | 0.99 | 3.6 |
| Rats | 75 | 7 | 0 | 4.21[b] | 1.1 |
| Dogs | 75 | 1 | 1.85 | 0 | --- |
| Dogs | 75 | 7 | 6.65 | 0.39 | 1.93 |
| Rats | 50 | 7 | 0 | 1.74[b] | 0.25 |
| Dogs | 50 | 1 | trace | 0.55 | --- |
| Dogs | 50 | 7 | 0.51 | 0.80 | 1.47 |
| Rhesus monkey | 75 | 3 | 3.70 | 1.67 | --- |
| Rhesus monkey | 75 | 7 | 8.72 | 7.98 | 4.12 |

[a] Zero exposure levels are subtracted from the values presented.

[b] Determined in plasma.

blood of all exposed animals, with the highest concentrations found in the Rhesus monkey (4.8 μmol/100 ml) and the dog (2.7 μmol/100 ml) following a 7-hour exposure to 75 ppm acrylonitrile. There was no detectable cyanmethemoglobin in the blood of rats at this exposure level; however, traces were detected at the 100 ppm exposure level. Brieger *et al*. (1952) concluded that acrylonitrile must be metabolized to cyanide and they suggested the possibility that a species' ability to detoxify cyanide was related to its resistance to acrylonitrile poisoning.

Several antidotes to cyanide poisoning have been evaluated for their effectiveness following acrylonitrile exposure, and as an indication of whether acrylonitrile has a mechanism of action similar to that of cyanide. Two types of antidotes have been used. The first, sodium thiosulfate, facilitates the detoxification of cyanide to thiocyanate by the following reaction:

$$\text{Thiosulfate} + \text{cyanide} \xrightleftharpoons[\text{thiocyanate oxidase}]{\text{rhodanese}} \text{sulfite} + \text{thiocyanate}$$

The second type consists of chemicals (methylene blue and nitrites) that cause the formation of methemoglobin, which competes with the cytochromes for the cyanide ion. The use of both types of antidotes has indicated that acrylonitrile toxicity cannot be attributed solely to the release of cyanide, and that either acrylonitrile or another metabolite of acrylonitrile has significantly toxic properties.

Hashimoto and Kanai (1965) examined blood levels of acrylonitrile, thiocyanate, and cyanide in rats, guinea pigs, and rabbits after intravenous (rabbits) or intraperitoneal (rats and guinea pigs) injection of acrylonitrile or of sodium thiosulfate followed in three minutes by acrylonitrile. As illustrated in Figures 13-3 and 13-4, in the rabbit, thiosulfate pretreatment did not affect the levels of acrylonitrile in the blood, but thiosulfate did cause a dramatic decrease in the cyanide levels and an increase in thiocyanate levels.

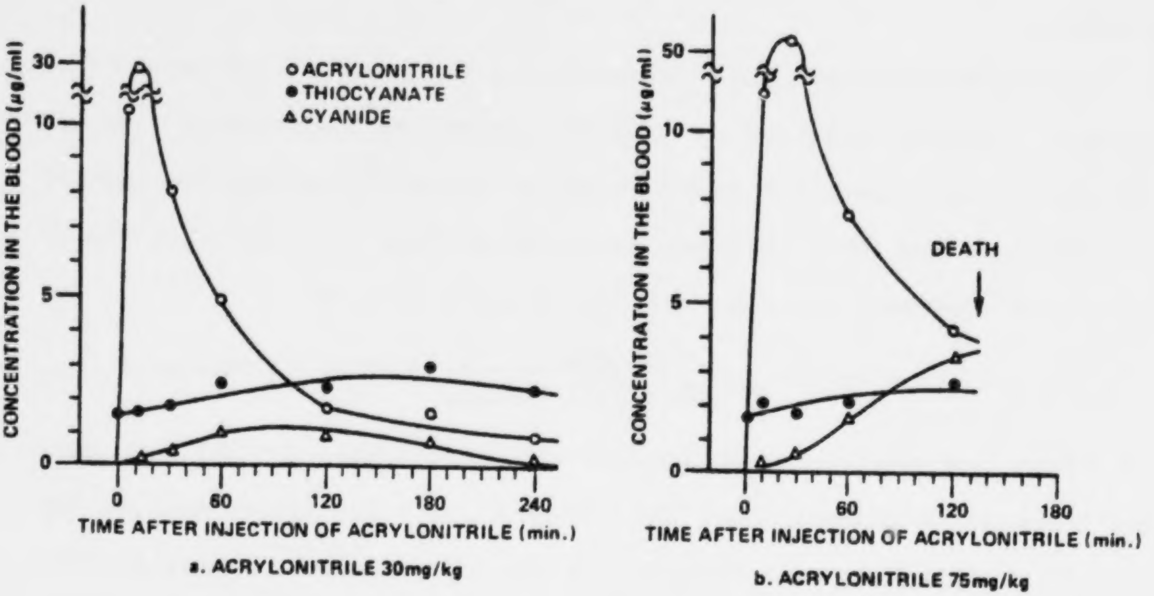
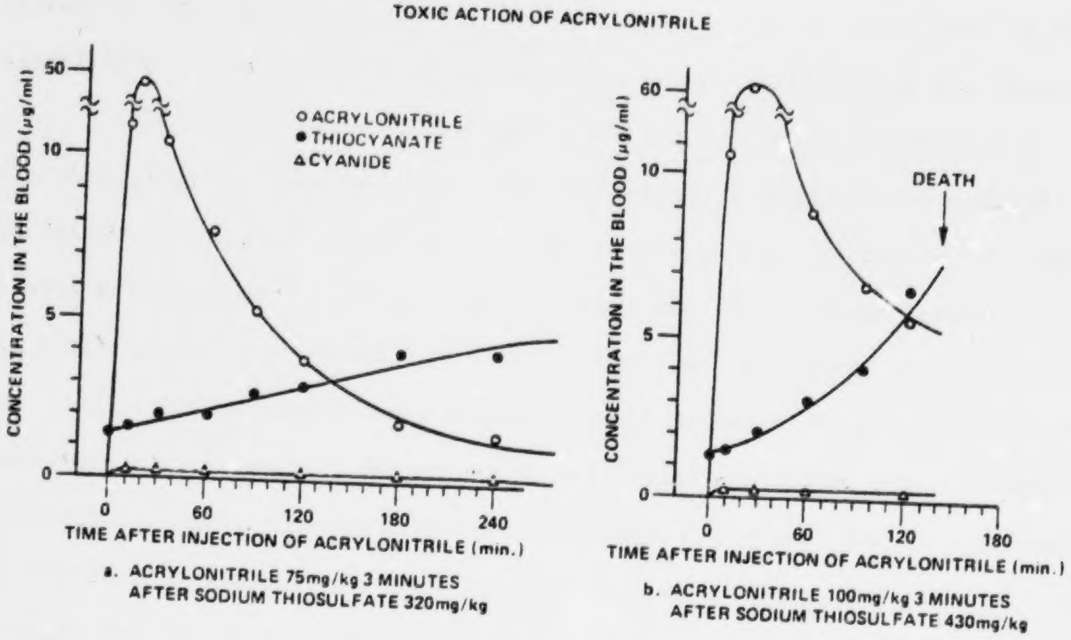


Figure 13-3. Distribution of Acrylonitrile, Cyanide, and Thiocyanate in the Blood after a Single Injection of Acrylonitrile (Rabbit) (Hashimoto and Kanai, 1965)



a. ACRYLONITRILE 75mg/kg 3 MINUTES AFTER SODIUM THIOSULFATE 320mg/kg

b. ACRYLONITRILE 100mg/kg 3 MINUTES AFTER SODIUM THIOSULFATE 430mg/kg

Figure 13-4. Effect of Sodium Thiosulfate on the Distribution of Acrylonitrile, Cyanide, and Thiosulfate (Rabbit) (Hashimoto and Kanai, 1965)

The use of thiosulfate prevented the death of three of the four rabbits exposed to 75 mg/kg acrylonitrile and reduced the severity of the symptoms of intoxication. At 100 mg/kg, death was delayed by thiosulfate, but all animals eventually succumbed to the toxic effects of acrylonitrile, even though the cyanide levels present in the blood were lower than those in animals that survived a 30 mg/kg exposure to acrylonitrile alone. Similar results were obtained using rats and guinea pigs, with the animals dying in spite of reduced blood cyanide levels (2.62 to 0.48 µg/ml in guinea pigs and 3.01 to 0.51 µg/ml in rats) following treatment with thiosulfate. It was apparent that even with extensive detoxification of liberated cyanide, acrylonitrile lost little of its toxicity.

Magos (1962) attempted to prevent the toxic effects of subcutaneously injected acrylonitrile by pretreating rats with an intraperitoneal injection of sodium nitrite. The results are shown in Table 13-10. Sodium nitrite effectively protected rats from the lethal effects of cyanide, but had no effect on either survival rate or survival time of animals treated with acrylonitrile. An estimation of the percentage inhibition of cytochrome oxidase in rats receiving cyanide and in rats receiving acrylonitrile revealed greater inhibition (84%) in rats that survived cyanide poisoning than in rats (71.5%) that were killed by acrylonitrile. From these results, it appeared that cyanide did not play a significant role in the toxic effects of acrylonitrile in rats unless the formation of cyanide from acrylonitrile in the tissue of exposed animals allows toxicity at a lower cyanide concentration.

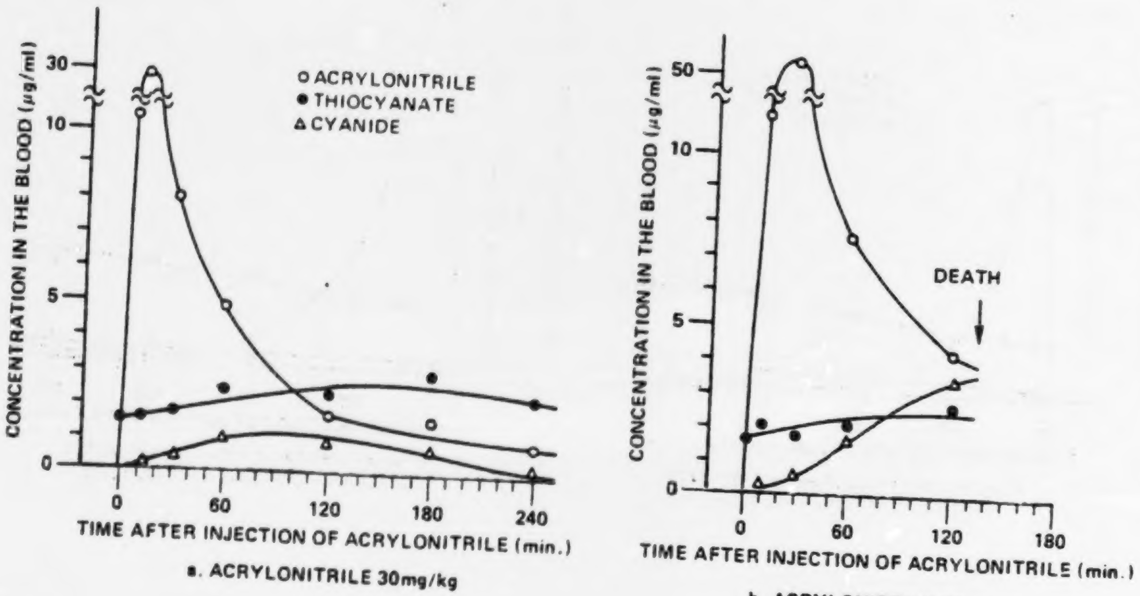
Benes and Cerna (1959) treated mice and rats orally with both sodium nitrite and thiosulfate and observed changes in the lethality of a subsequent oral dose of acrylonitrile. In rats, the cyanide antidotes protected 30% of the animals from a LD100 dose of acrylonitrile. In mice, the effect was more dramatic, with protection afforded at up to three times the LD50 dose. It was concluded that

Table 13-10. Effect of Methemoglobinemia on Mortality Ratios in Albino Rats Poisoned with Acrylonitrile, Potassium Cyanide, and Acetone Cyanohydrin (Magos, 1962)

| Compound | Dose (millimole/kg) | No. That Died/No. Given Cyanide Compound | |
|---|---|---|---|
| | | Without Sodium Nitrite | With Sodium Nitrite |
| Acrylonitrile | 2.8 | 5/5 | 5/5 |
| Potassium cyanide | $2.4 \times 10^{-1}$ | 5/5 | 1/5 |
| Potassium cyanide | $3.7 \times 10^{-1}$ | -- | 4/4 |
| Acetone cyanohydrin | $1.6 \times 10^{-1}$ | 5/5 | 0/5 |

toxicity in mice might be due to cyanide, while in rats the toxicity is due mainly to the acrylonitrile molecule itself. It was shown that by 3 days post exposure, rats had metabolized only 19.4% of the acrylonitrile to cyanide (the data for mice were not given). Benes and Cerna (1959) suggested that species variation in susceptibility to acrylonitrile intoxication might be due to the ability of animals to metabolize acrylonitrile to cyanide. Other investigators (Magos, 1962; Gut et al., 1975), however, suggested that the difference was due to differences in ability to detoxify the liberated cyanide. The exact role of liberated cyanide both in the toxicity of acrylonitrile and in the almost tenfold difference in susceptibility of experimental animals to acrylonitrile's toxic effects is still unclear.

In further studies of the mechanism of acrylonitrile's toxicity, Hashimoto and Kanai (1965) pretreated rabbits intravenously with L-cysteine 3 minutes prior to dosing with acrylonitrile. It was shown in a test tube reaction that L-cysteine forms an addition product with acrylonitrile by cyanoethylation, and Gut et al. (1975) demonstrated that S-(2-cyanoethyl)-cysteine was non-toxic and not metabolized to thiocyanate when administered orally to rats. Hashimoto and Kanai (1965) analyzed the blood of rabbits treated with acrylonitrile and with acrylonitrile and cysteine, and found a large decrease in the levels of acrylonitrile and cyanide in animals treated with cysteine (Figures 13-5 and 13-6). Unlike the antidotes to cyanide poisoning, cysteine not only relieved the symptoms of poisoning, but rabbits receiving 100 mg/kg acrylonitrile also survived and completely recovered from the treatment. Similar results were noted with guinea pigs, rats, and mice (Table 13-11). Positive effects were noted with other sulfur-containing compounds. Because cysteine could protect animals from the toxic effects of acrylonitrile by reacting directly with the acrylonitrile molecule, and cyanide antidotes were not effective in preventing death,



a. ACRYLONITRILE 30mg/kg

b. ACRYLONITRILE 75mg/kg

Figure 13-5. Distribution of Acrylonitrile, Cyanide, and Thiocyanate in the Blood after a Single Injection of Acrylonitrile (Rabbit) (Hashimoto and Kanai, 1965)

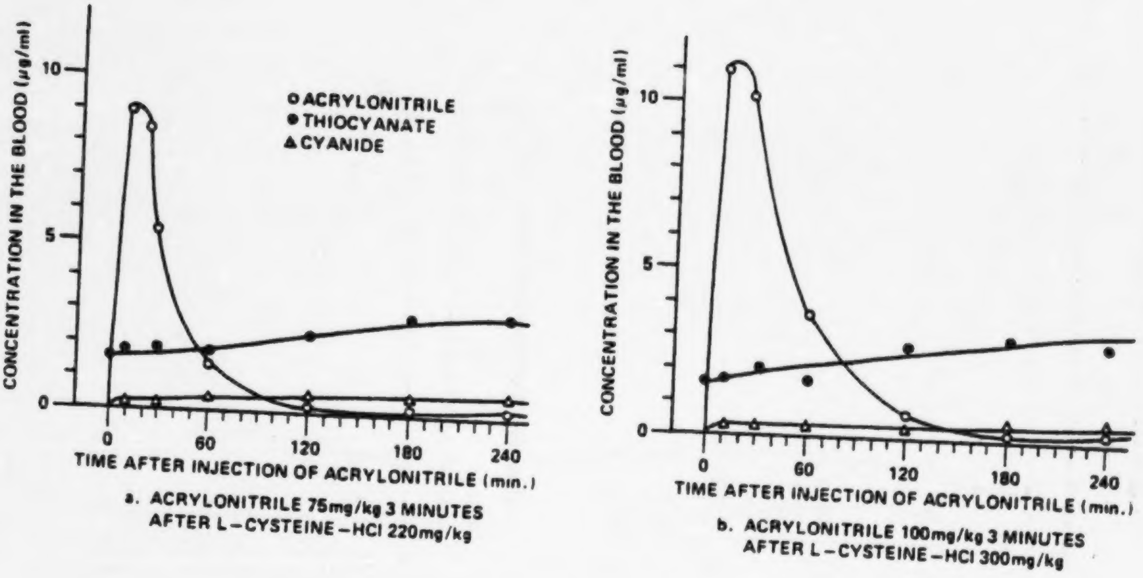


Figure 13-6. Effect of L-Cysteine on the Blood Concentrations of Acrylonitrile, Cyanide, and Thiocyanate (Rabbit) (Hashimoto and Kanai, 1965)

Table 13-11. Therapeutic Effect of SH and S-S Compounds on Acute Acrylonitrile Poisoning[a] (Hashimoto and Kanai, 1965)

| Animal<br>Amount of Acrylonitrile Injection<br>Site of Injection | Control | | L-Cysteine HCl 300=370 mg/kg | | L-Glutathione 570 mg/kg | | α-Mercapto-propionylglycine 350 mg/kg | | L-Methionine 425 mg/kg | |
|---|---|---|---|---|---|---|---|---|---|---|
| | survival rate | survival time (min) | survival rate | survival time (min) | survival rate | survival time (min) | survival rate | survival time (min) | survival rate | survival time (min) |
| Rabbit (5)[b]<br>100 mg/kg<br>iv | 0/5 | 98(5) | 5/5 | -[c](5) | 5/5 | -c(5) | 4/5 | 100(1) | 5/5 | (5) |
| Guinea pig (5)<br>125 mg/kg<br>ip | 0/5 | 86(5) | 2/5 | 132(3) | ---[d] | --- | --- | --- | --- | --- |
| Rat (5)<br>100 mg/kg<br>ip | 0/5 | 85(5) | 2/5 | 254(3) | --- | --- | --- | --- | --- | --- |
| Mouse (5)<br>100 mg/kg<br>ip | 0/5 | 101(5) | 5/5 | -[c](5) | --- | --- | --- | --- | --- | --- |

[a] Amount of AN = 2 x LD50, amount of SH and S-S compounds = AN x 1 =1.5 equivalent.

[b] All numbers in parentheses represent number of animals.

[c] Animals survived treatment

[d] --- = Animals not treated

Hashimoto and Kanai (1965) speculated that acrylonitrile itself was a toxic agent and may act in conjunction with cyanide to produce the ultimately toxic effects. Pilotti (1975) found no acrylonitrile toxicity in vitro, however, as indicated by inhibition of growth of ascites sarcoma BP8 cells in culture (1.0 mmol acrylonitrile was assayed) and attributed this to the lack of formation of toxic metabolites. It is uncertain whether the toxic activity ascribed to acrylonitrile is due to the acrylonitrile molecule per se or to a non-cyanide metabolite of acrylonitrile.

Acrylonitrile also reacts with endogenous glutathione by either one or both of two possible mechanisms. Direct interaction may occur by S-cyanoethylation of glutathione, or alternatively by enzymatic conjugation with glutathione during the detoxification of acrylonitrile. Dinu (1975b) demonstrated dramatic decreases in non-protein tissue thiol concentration (protein thiol levels were unchanged) in rats following subcutaneous injection of two times the LD50 dose of acrylonitrile (Table 13-12). Similar results were observed by Szabo et al. (1977a, 1977b), who used direct measurements of tissue glutathione. The possibility that glutathione levels were important in mediating the toxicity of acrylonitrile was indicated by the greater sensitivity of rats to the toxic effects of acrylonitrile at night when liver glutathione levels were low due to normal diurnal variation (Jaeger, 1979). Also, Szabo et al. (1977a) showed a correlation between the doses of acrylonitrile that caused a precipitous decline in glutathione concentrations in the brain of rats and the doses that caused mortality; however, they noted no tissue damage by light microscopy in the liver, lung, or kidney of rats 60 minutes after an intravenously administered dose (15 mg/100 g body weight) of acrylonitrile, even though the glutathione levels were reduced by 80 to 90%. Dinu (1975a) presented evidence that low glutathione levels caused by acrylonitrile lead to excess formation of lipid

Table 13-12. Concentration of Protein (PBSH) and Nonprotein (NPSH) SH Groups in Normal and Acrylonitrile-Intoxicated Animals (µmoles SH/100 g wet tissue) (Dinu, 1975b)

| | Normal Animals Mean ± Standard Error | Intoxicated Animals Mean ± Standard Error |
|---|---|---|
| **Liver** | | |
| PBSH | 1604 ± 28.3 | 1524 ± 46.3 |
| NPSH | 412 ± 16.8 | 42.4 ± 1 |
| **Kidney** | | |
| PBSH | 952 ± 40 | 900 ± 26.3 |
| NPSH | 250 ± 40 | 24.6 ± 0.6 |
| **Brains** | | |
| PBSH | 968 ± 27.4 | 836 ± 26 |
| NPSH | 103 ± 3.55 | 34 ± 1.48 |
| **Lung** | | |
| PBSH | 989 ± 30 | 864 ± 24.4 |
| NPSH | 82 ± 3 | 28 ± 1.48 |
| **Testis** | | |
| PBSH | 1070 ± 41 | 1000 ± 39 |
| NPSH | 237 ± 3.6 | 86 ± 4.9 |
| **Adrenal** | | |
| PBSH | 528 ± 20.7 | 538 ± 11 |
| NPSH | 127 ± 4 | 0 ± --- |

peroxides and subsequent disruption of membrane function. Following treatment of rats with acrylonitrile (two times the LD50 dose administered subcutaneously), increases in malonaldehyde were observed in the liver. According to Dahle et al. (1962), the formation of malonaldehyde parallels the formation of lipid peroxides. The increase in lipid peroxides occurred even though the treatment with acrylonitrile stimulated the activity of glutathione peroxidase. The low levels of glutathione in intoxicated animals made the protective effects of glutathione peroxidase ineffective. Although comment was made on the disruptive properties of lipid peroxides on membrane function, no measurements of membrane function were made.

Hashimoto and Kanai (1965) examined respiration in guinea pig brain and liver slices incubated with acrylonitrile ($10^{-3}$ M). The oxygen consumption of the brain slices was measured under both potassium stimulating and non-potassium stimulating conditions. Acrylonitrile caused a 20% decrease in the oxygen usage of the potassium stimulated preparation, while no inhibition was noted in the non-potassium stimulated preparation. This inhibition of respiration appeared to be specific to the brain slice. Inhibition of respiration in liver slices was not observed until $5 \times 10^{-2}$ M acrylonitrile was used, at which point precipitation of protein occurred in the liver. There was no protection offered by the simultaneous administration of sodium thiosulfate with acrylonitrile. It was also apparent that inhibition of respiration was not due to the liberation of cyanide, since cyanide at $10^{-4}$ M inhibited both potassium stimulated and non-stimulated brain slices, and sodium thiosulfate protected the brain slices from the detrimental effects of cyanide. Effects on the peripheral nerves were demonstrated by applying acrylonitrile to the nerve trunk in an isolated Sciatic-gastrocnemius preparation from frog and observing a rapid anesthetic action. This anesthetic effect rapidly disappeared following removal of acrylonitrile.

It was concluded that acrylonitrile produces abnormal function of both the peripheral and central nervous systems.

Acute adrenal apoplexy has been described in rats following the administration of acrylonitrile (Szabo _et_ _al_., 1976b). Following intravenous administration of 15 mg acrylonitrile/100 g body weight, the lesions appeared bilaterally between 1 and 2 hours after treatment. The intravenous administration of this dose produced hemorrhage in 90 to 100% of the animals, while administration of the same dose of acrylonitrile by gavage produced hemorrhage in only 20% of the animals. As reported by Szabo and Selye (1971), mortality in both cases was 100%. Szabo _et_ _al_. (1977b) observed early hematological changes consistent with a longer blood clotting time in rats following the intravenous administration of acrylonitrile, but it was not clear if these hematological changes were sufficient in themselves to cause the hemorrhage. Protection from adrenal apoplexy and, to some degree, mortality was offered by pretreating the animals for 4 days with phenobarbital and some steroids. It was believed that this protection was offered by the ability of these xenobiotics to induce drug-metabolizing enzymes and hence alter the metabolism of acrylonitrile (Szabo and Selye, 1972). Since mortality was not always associated with adrenal apoplexy, it was not apparent how this hemorrhage effect related to the systemic toxicity of acrylonitrile.

### 13.2.1.4 Acute Topical Irritation and Toxicity

McOmie (1943) applied acrylonitrile to the shaved abdomens of rabbits at doses of 1.0, 2.0, and 3.0 ml/kg to areas of 100, 200, and 200 $cm^2$, respectively. No attempt was made to prevent evaporation of the test compound. Slight vasodilatation was observed at the low dose, while the two higher doses caused slight erythema. Only at the highest dose was systemic toxicity noted, as indicated by depression and increased respiration rate. The animal showing toxic signs recovered without any therapeutic treatment. When acrylonitrile was kept in contact with the abraded skin of rabbits by absorption into a cellulose pad, the LD50 was 0.25 ml/kg (Roudabush et al., 1965). When guinea pigs were used, the LD50 for animals with abraded skin was 0.86 ml/kg, while the LD50 for animals with intact skin was 0.46 ml/kg. It is interesting to note that in the guinea pig, toxicity was greater for acrylonitrile when applied to intact skin.

Acrylonitrile caused only mild irritation to the eye of a rabbit when applied in a single 0.05 ml drop (McOmie, 1943). One hour following treatment, mild conjunctivitis was observed with no clouding of the cornea or pupillary damage. The animal had completely recovered by 24 hours.

Skin irritation as a result of contact with acrylonitrile has also been observed in humans (Wilson et al., 1948). When the skin was exposed to acrylonitrile vapors only, workers complained of itching although there was no observable dermatitis. When the skin was in contact with the liquid, the symptoms included irritation, erythema followed by the formation of blebs, desquamation, and slow healing. Dudley and Neal (1942) reported on a laboratory accident in which a small quantity of acrylonitrile was spilled on the hands of an individual. The symptoms of diffuse erythema occurred by 24 hours, with blisters on the finger tips and slight swelling. Erythematous and painful itching occurred by the third day. Ten days after exposure, the skin was cracked and peeled and large areas of tender new skin were present. No cases of human fatality have been directly associated with absorption of acrylonitrile through the skin.

### 13.2.2 Subchronic Toxicity in Non-Human Mammals

In a preliminary study, Dudley et al. (1942) exposed 4 rhesus monkeys and 2 dogs to an atmosphere containing 56 ppm (122 mg/m$^3$) acrylonitrile for 4 hours a day, 5 days a week, over a 4-week period. The rhesus monkeys showed no overt signs of toxicity. Of the two dogs treated, one died during the first exposure period and the second had intermittent paralysis of the hind legs following three of the exposure periods (the fifth, thirteenth, and fourteenth). In subsequent experiments, 16 rats, 16 guinea pigs, 3 rabbits, and 4 cats were exposed to 100 ppm (217 mg/m$^3$) acrylonitrile, and 16 rats, 16 guinea pigs, 4 rabbits, 4 cats, and 2 rhesus monkeys were exposed to 153 ppm (332 mg/m$^3$) acrylonitrile. The exposure conditions were similar to those described above except the period was increased to 8 weeks. At the 100 ppm (217 mg/m$^3$) level, rats and guinea pigs showed only slight lethargy. Three female rats gave birth to normal pups during the study. The rabbits did not gain weight during the experiment, and the cats suffered the most severe symptoms of vomiting and loss of weight. At the highest exposure levels, severe toxicity was noted in all animals, with many dying prior to the completion of the study; symptoms were similar to those noted after acute exposure to acrylonitrile. In general, species sensitivity to subchronic acrylonitrile intoxication was similar to that observed following acute exposure, with the dog being the most sensitive and rats and guinea pigs the least sensitive to acrylonitrile.

Dudley _et al_. (1942) examined the spleen, kidneys, liver, lungs, heart, pancreas, lymph nodes, stomach, and small and large intestines of 18 rats, 6 rabbits, 6 cats, 16 guinea pigs, and 1 monkey for pathological changes (there was no explanation of how the animals were selected for examination). The spleen of rats showed hemosiderosis, which is indicative of blood destruction. The kidneys of most animals showed renal irritation, with hyaline casts in animals in the two higher exposure groups (the exceptions were 1 cat and 1 rabbit exposed at the 100 ppm level and 1 monkey exposed at the 153 ppm level). Interstitial nephritis, although not very extensive, was also noted in many animals (the exception being the monkey and rats exposed at the 100 ppm level). Subacute bronchopneumonia was present in at least some members of all the species studied except the cat; the pneumonia appeared as congestion and edema in the alveoli. Cats were the only species that showed signs of liver damage, although the exact lesions were not described.

Hematological determinations in rats and rabbits were made by Dudley and coworkers (1942) using animals that had been exposed to 153 ppm (332 $mg/m^3$) acrylonitrile. These determinations revealed no changes in red and white blood cell counts or hemoglobin levels. At the end of the first week of exposure, an increase in eosinophils was noted using differential cell counts, with the maximum for rabbits and rats being 42% and 21% above control, respectively. Minami et al. (1973) claimed that changes in the partial pressures of oxygen and carbon dioxide in the blood occurred, along with increases in hemoglobin and hematocrit values following exposure of 8 rabbits once a week for 8 hours to 20 ppm (43 $mg/m^3$) acrylonitrile for a period of 8 weeks. Due to the small differences observed and the relatively large standard deviations, however, the statistical significance of these conclusions is in doubt.

Some cumulative effects were observed in rats that received 50 mg/kg acrylonitrile by daily intraperitoneal injection for a 3-week period (Knoblock et al., 1971). The animals lost body weight, but the liver, kidneys, and heart increased in weight. Signs of functional disorders of both liver and kidneys were noted, and microscopic examination showed slight damage in neuronal cells of the brain cortex, brain stem, and in the parenchymal cells of the liver. The only hematological change noted was leukocytosis.

In an abstract, Szabo and coworkers (1976a) reported subacute effects of acrylonitrile in female Sprague-Dawley rats (200 g) exposed to the compound at 2000 ppm in the drinking water for 21 days. At this concentration of acrylonitrile, water intake was reduced in the experimental animals to one-third of that of the control animals. Limited pathological studies indicated atrophy of the zona fasciculata and increases in the zona glomerulosa of the adrenals. Elevated plasma $Na^+$ concentration of 9 mEq/l ($K^+$ was not changed) and reduced corticosterone concentrations of 24 μg (the denominator was not indicated) were noted. The authors concluded that acrylonitrile affected the mineral-corticoid and glucocorticoid cells of the adrenal cortex. Using the same strain of rats (Sprague-Dawley), Szabo et al. (1977b) demonstrated a dose-dependent increase in glutathione levels following a 21-day exposure to water containing 20, 100, or 500 ppm acrylonitrile. It was suggested that this increase in glutathione was due to a rebound effect following the utilization of glutathione in the detoxification of acrylonitrile.

Because of the limited number of studies on subchronic exposure to acrylonitrile, it is difficult to formulate a unified pattern of toxic action, both in terms of the mechanism of toxicity and the target organs. Although a number of biological parameters have been reported to change following subacute exposure to acrylonitrile, it is not clear how these effects relate to the primary toxic action of acrylonitrile.

### 13.2.3 Chronic Toxicity in Non-Human Mammals

Two chronic toxicity studies have been conducted by the Dow Chemical Toxicology Research Laboratory for the Chemical Manufacturers Association. In both studies, the animals were exposed by ingesting drinking water containing acrylonitrile. The animals were frequently evaluated during the studies for food and water consumption, weight gain, and blood and urine chemistry. On death or at necropsy at the end of the study, autopsies were performed in order to detect gross or microscopic pathological conditions.

In the first study (Quast et al., 1975), beagle dogs (four dogs of each sex and exposure level) were exposed to 300, 200, and 100 ppm acrylonitrile for a 6-month period starting when the dogs were 8 months old. By using water consumption data, the mean doses of acrylonitrile ingested at the three exposure levels were for male dogs 17 ± 4, 16 ± 2, and 10 ± 1 mg/kg/day, respectively, and for female dogs 18 ± 5, 17 ± 2, and 8 ± 1 mg/kg/day, respectively. The two highest exposure levels (300 and 200 ppm) were highly toxic to the dogs; 3 of the 4 males and 2 of the 4 females at the 300 ppm level and 2 of the 4 males and 3 of the 4 females at the 200 ppm levels either died spontaneously or were euthanized because of their debilitated state prior to three months (the ingested dose of acrylonitrile at these exposure levels was nearly identical). Two male dogs at the 300 ppm exposure level were removed from the study at 57 days because of signs of overt toxicity and were placed on water containing no acrylonitrile. These dogs appeared to recover completely (they regained their pretest weight) and, after removal from acrylonitrile exposure for approximately 1 month, they were returned to the study. Subsequently, the dogs again showed signs of toxicity and died within 24 and 45 days. All dogs that died showed the following progression of signs: terminal depression, lethargy, weakness, emaciation, and respiratory distress. Dogs that ingested water containing 100 ppm acrylonitrile and dogs that survived treatment at the higher doses appeared normal during the period of study.

The presence of acrylonitrile in the drinking water caused a significant decrease in water and food consumption in both male and female dogs at the 300 ppm level. At the other exposure levels, male dogs showed normal water and food consumption, while female dogs had sporadically lower water consumption and consistently lower food consumption. A supplemental study, which used 8 female dogs that ingested water containing 100 ppm acrylonitrile, failed to demonstrate any decrease in either food or water consumption. With regard to general weight gains (only surviving animals were considered since emaciation was a symptom ensued by morbidity), all male dogs gained weight, although less than that gained by control animals, while female dogs lost weight, except for the animals at the 100 ppm exposure levels. It was not clear whether the unpalatability of water containing acrylonitrile may have affected these results.

Chemical studies on blood and urine samples revealed no significant abnormalities that were associated directly with acrylonitrile treatment. Some hematologic changes, however, did occur at the higher exposure levels. These changes were noted and were consistent with bronchopneumonia, which was chronically present (and the reason for death) in the high dosed animals. Analysis of the liver and kidneys for non-protein sulfhydryl content revealed no statistical differences in the animals at the 100 ppm exposure levels and no apparent effect at other doses, although these data could not be analyzed statistically because of the small group size. There was also no difference in the immunologic status of treated animals as assessed by percent of albumin, alpha 1, alpha 2, beta, and gamma globulin in the serum.

On autopsy, some minor or indirectly-caused pathological findings were noted. In male dogs at the 100 and 200 ppm exposure levels, there was an increase in the relative kidney to body weight ratio (there were no changes in the single male dog exposed to 300 ppm acrylonitrile). The kidney appeared normal on histological examination and the weight gain may have been compensatory. There was also a decrease in the relative brain weight of the 2 surviving male dogs at the 200 ppm exposure level and an increase in the relative brain weight of the 2 surviving female dogs at the 300 ppm exposure level. No mention was made of results from histological examination of these brains. The lungs of dogs at the higher exposure levels showed typical pathologies of foreign body bronchopneumonia, the cause of which appeared to be aspirated food particles. The only treatment-related pathology was dilation, thinning of the walls, and focal erosions or ulcerations of the esophagus in male and female dogs in the 300 and

200 ppm exposure groups. In some animals, although not consistently, there was a thickening of the epithelium lining of the dorsal surface of the tongue. It was believed that acrylonitrile may have caused irritation of the membrane of the throat, resulting in the aspiration of food particles.

In the second study conducted by Dow Chemical Company, Quast et al. (1980a) exposed Sprague-Dawley rats to drinking water containing 35, 85, and 210 ppm acrylonitrile. After the first 21 days the concentrations of acrylonitrile were changed to 35, 100, and 300 ppm. The calculated amount of acrylonitrile ingested was 3.42, 8.53, and 21.18 mg/kg/day for male rats and 4.36, 10.76, and 24.97 mg/kg/day for female rats. By 9 months of treatment, the animals at the two highest dose levels had rough hair coats and an unthrifty appearance. There was a treatment-related early mortality in female rats at all exposure levels and in male rats at the highest exposure levels (Table 13-13). The differences in males and females may be partly related to the larger doses of acrylonitrile ingested by female rats.

There was a significant dose-related decrease in daily water consumption of both male and female rats exposed to acrylonitrile. This decrease in water consumption was probably due to voluntary restriction of intake as a result of an unpalatable taste imparted by acrylonitrile. The lower water consumption in treated rats was the probable cause of the concomitant decrease in food consumption and body weight. Chronic renal disease, which was common in older rats of this strain, occurred less frequently in treated rats probably as a result of the lower consumption of food and water. With this decrease in renal disease there was a decrease in both gross and microscopic pathologies and clinical findings associated with renal failure. Decreased food and water consumption resulted in emaciation of the animals, which caused other clinical signs and pathologies to change.

Table 13-13. Cumulative Mortality of Male and Female Rats Maintained for Two Years on Drinking Water Containing Acrylonitrile (Quast _et al._, 1980a)

| Days on Test | Percent Dead | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | 35 ppm | | 100 ppm | | 300 ppm | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| 0-30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31-60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 61-90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 91-120 | 0 | 0 | 0 | 0 | 2.1 | 0 | 0 | 0 |
| 121-150 | 0 | 0 | 0 | 0 | 2.1 | 0 | 0 | 2.1 |
| 151-180 | 1.3 | 0 | 0 | 0 | 2.1 | 0 | 0 | 2.1 |
| 181-210 | 1.3 | 1.3 | 0 | 0 | 2.1 | 0 | 0 | 2.1 |
| 211-240 | 1.3 | 1.3 | 2.1 | 0 | 2.1 | 2.1 | 0 | 2.1 |
| 241-270 | 2.5 | 1.3 | 2.1 | 0 | 2.1 | 2.1 | 4.2 | 6.3 |
| 271-300 | 3.8 | 1.3 | 2.1 | 0 | 2.1 | 4.2 | 4.2 | 8.3 |
| 301-330 | 7.5 | 1.3 | 2.1 | 0 | 2.1 | 6.3 | 4.2 | 18.8[a] |
| 331-360 | 8.8 | 1.3 | 4.3 | 2.1 | 2.1 | 6.3 | 8.3 | 29.2[a] |
| 361-390 | 8.8 | 1.3 | 4.3 | 2.1 | 2.1 | 6.3 | 14.6 | 33.3[a] |
| 391-420 | 10.0 | 3.8 | 6.4 | 6.3 | 4.2 | 10.4[a] | 16.7 | 41.7[a] |
| 421-450 | 13.8 | 3.8 | 10.6 | 6.3 | 10.4 | 12.5 | 22.9 | 50.0[a] |
| 451-480 | 18.8 | 7.5 | 14.9 | 10.4 | 14.6 | 12.5 | 31.3 | 64.6[a] |
| 481-510 | 26.3 | 11.3 | 17.0 | 14.6 | 22.9 | 22.9[a] | 45.8[a] | 72.9[a] |
| 511-540 | 36.3 | 13.8 | 27.7 | 20.8 | 33.3 | 27.1[a] | 60.4[a] | 77.1[a] |
| 541-570 | 41.3 | 22.5 | 29.8 | 25.0 | 37.5 | 35.4[a] | 66.7[a] | 87.5[a] |
| 571-600 | 50.0 | 27.5 | 42.6 | 41.7[a] | 47.9 | 56.3[a] | 70.8[a] | 93.8[a] |
| 601-630 | 60.0 | 42.5 | 48.9 | 50.0[a] | 54.2 | 70.8[a] | 77.1[a] | 95.8[a] |
| 631-660 | 67.5 | 46.3 | 57.4 | 56.3 | 68.8 | 79.2[a] | 83.3[a] | 95.8[a] |
| 661-690 | 83.8 | 56.3 | 70.2 | 68.8[a] | 72.9 | 87.5[a] | 100.0[a] | 100.0[a] |
| 691-720 | 87.5 | 67.5 | 83.0 | 79.2[a] | 83.3 | 91.7[a] | 100.0[a] | 100.0[a] |
| 721-745 | 91.3 | 75.0 | 87.2 | 87.5[a] | 89.6 | 95.8[a] | 100.0[a] | 100.0[a] |
| | | | 89.4 | 91.7[a] | | 97.9[a] | | |
| Total Number of Rats | 80 | 80 | 47 | 48 | 48 | 48 | 48 | 48 |

[a] Significantly different from controls by Fisher's Exact Probability Test, $p < 0.05$.

Most of the clinical chemistry and pathological changes that occurred at a statistically significant level ($P < 0.05$) were attributed to secondary effects of treatment and were not considered to be caused by a toxic action of acrylonitrile. A summary of both the clinical chemistry and pathological changes observed is presented in Table 13-14. Although it was proposed that these changes were, for the most part, a result of restricted food and water consumption, it was impossible to state this unequivocally because of the lack of pair-fed controls. There were no severe or irreversible non-tumorigenic pathological findings. The only non-tumorigenic changes that were considered to be treatment-related were of a possible pre-neoplastic type; these include hyperplasia and hyperkeratosis of the squamous epithelium of the nonglandular portion of the stomach (100 and 300 ppm males and all treatment levels in females), proliferation of glial cells in the brain (300 ppm males and 35 and 100 ppm females), and mammary gland hyperplasia (300 ppm females). To fully understand the chronic toxicity of acrylonitrile, studies must be performed with pair-fed controls that are in the same nutritional state as the experimental animals, and evaluation of clinical signs must be made in the absence of gross neoplasias.

13.2.4 Summary and Conclusions - Acrylonitrile intoxication in humans results in irritation of the eyes and nose, weakness, labored breathing, dizziness, impaired judgement, cyanosis, nausea, and convulsions. The TLV for acrylonitrile is 4.5 $mg/m^3$ (2 ppm) for humans. Acrylonitrile also causes severe burns to the skin. In experimental animals, there is considerable species variation in susceptibility to acrylonitrile intoxication; the guinea pig is the most resistant and the dog is the most sensitive. In animals, effects of intoxication include respiratory changes, cyanosis, convulsions, and death. In rats, the $LD_{50}$ for acrylonitrile is between 80 and 113 mg/kg (Knoblock _et al_., 1971; Smyth _et al_., 1969). There is some evidence that acrylonitrile produces abnormal function

Table 13-14. Significant Changes Considered to be Secondary to Ingestion of Acrylonitrile (Quast et al., 1980a)

| Type of Analyses | Significant Findings | Suspected Cause of Findings |
|---|---|---|
| Hematology | Decreased white blood cell count<br>Decreased packed cell volume<br>Decreased hemoglobin | Blood loss from ulcerated tumors |
| Urinalysis | Increased urine specific gravity | Restricted water consumption |
| Clinical Chemistry | Increased blood urea nitrogen | Restricted water and food consumption |
| | Decreased serum glutamic pyruvic transaminase | Within normal limits |
| Pathology | Emaciation<br>Decreased adipose tissue | Restricted water and food consumption |
| | Dental malocclusion | Ear canal tumors deviating the lower jaw |
| | Increased blood and bile-like fluid | Restricted food consumption and ulcerating tumors |
| | Decreased chronic renal disease | Restricted food and water consumption and early death |
| | Increased hepatocellular atrophy | Poor nutritional state |
| | Decreased signs of altered nervous system function | Decrease in chronic renal disease |
| | Changes in organ weight | Poor nutrition |

of both the peripheral and central nervous systems and that acrylonitrile causes damage to the adrenals. With subchronic exposure of animals to acrylonitrile, some signs of functional disorders of the liver and kidney are observed. Chronic exposure of dogs and rats results in unthrifty appearance, weight loss, and early death. Some of these signs may be related to low food and water consumption resulting from the unpleasant taste of acrylonitrile in the water. Pathological changes in the rats believed to be treatment-related included hyperplasia and hyperkeratosis of the squamous epithelium of the nonglandular portion of the stomach, proliferation of glial cells in the brain, and mammary gland hyperplasia in females.

## 13.3 TERATOGENICITY AND REPRODUCTIVE TOXICITY

The basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" were summarized by the Office of Pesticides and Toxic Substances (U.S. EPA, 1980c) as follows:

"Generally, the term 'teratogenic' is defined as the tendency to produce physical and/or functional defects in offspring *in utero*. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis, edema, and increased incidence of supernumerary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of the developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to in utero exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to 'catch up'. Unless a series of specific kidney function tests is performed on the neonates, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting after-effects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of embryonic/fetal effects in one species might, under a given agent, react in another species in a more serious and irreversible manner."

The teratogenicity of acrylonitrile has been investigated using Sprague-Dawley rats exposed to the compound by inhalation or by gavage on days 6 to 15 of gestation (the results of both the inhalation and gavage treatments have been reported by Murray et al., 1978, while a detailed report of the results of the gavage treatment was presented by Murray et al., 1976). Acrylonitrile was administered at doses of 10, 25, and 65 mg/kg/day per os or by exposing the animals to an atmosphere of 40 or 80 ppm (87 or 147 mg/$m^3$) acrylonitrile for 6 hours per day. It was estimated by measuring blood levels of acrylonitrile and its metabolites that an exposure to 80 ppm (174 mg/$m^3$) acrylonitrile for 6 hours was equivalent to a single 23 mg/kg dose of acrylonitrile. On day 21 of gestation, the dams were sacrificed and examined for implants and fetal abnormalities.

Rats exposed to 65 mg/kg/day showed moderate signs of toxicity with decreased body weight, thickening of the non-glandular portion of the stomach, and increased liver weight. The only adverse effect on animals exposed to 25 mg/kg/day was a slight thickening of the non-glandular portion of the stomach, while animals exposed to the lowest dose or animals exposed by inhalation showed no major treatment-related toxic effects. In all cases, except the animals treated with 10 mg/kg/day, food consumption initially declined during the first 3-day interval following treatment, but returned to normal during the two subsequent 3-day intervals. Neither the stress on the dams due to the mild toxic effects of acrylonitrile nor any direct toxic effects of acrylonitrile on the fetus significantly altered the number of litters, implants per dam, or live fetuses per litter. A slight but significant decrease in fetal body weight and crown-rump length was noted in the 65 mg/kg/day group, but not in any of the other groups.

Fetal malformations were observed primarily in the offspring of animals that received acrylonitrile by gavage (Table 13-15). Following inhalation of

Table 13-15. Incidence of Fetal Malformations Among Litters of Rats Given Acrylonitrile (Murray et al., 1978)

| Type of Malformation | Acrylonitrile given by gavage (mg/kg body weight/day) | | | | Acrylonitrile given by inhalation (ppm) | | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 25 | 65 | 0 | 40 | 80 |
| | No. fetuses/No. litters examined | | | | | | |
| External and skeletal | 443/38 | 388/35 | 312/29 | 212/17 | 421/33 | 441/36 | 406/35 |
| Visceral malformations | 154/38 | 135/35 | 111/29 | 71/17 | 140/33 | 148/36 | 136/55 |
| | No. of fetuses (litters) affected | | | | | | |
| External malformation | | | | | | | |
| Short tail[b] | 1(1) | 0(0) | 2(2) | 8(6)[a] | 0(0) | 0(0) | 2(2) |
| Short trunk[b] | 0(0) | 0(0) | 0(0) | 3(3)[a] | 0(0) | 0(0) | 1(1) |
| Imperforate anus[b] | 0(0) | 0(0) | 0(0) | 2(2) | 0(0) | 0(0) | 0(0) |
| Omphalocele | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1(1) | 1(1) |
| Visceral malformations | | | | | | | |
| Right-sided aortic arch | 0(0) | 0(0) | 1(1) | 1(1) | 0(0) | 0(0) | 0(0) |
| Missing kidney, unilateral | 1(1) | 0(0) | 0(0) | 1(1) | 0(0) | 0(0) | 0(0) |
| Anteriorly-displaced ovaries[b] | 0(0) | 0(0) | 1(1) | 1(1) | 0(0) | 0(00 | 1(1) |
| Skeletal malformations | | | | | | | |
| Missing vertebrae (associated with short tail)[b] | 1(1) | 0(0) | 2(2) | 8(6)[a] | 0(0) | 0(0) | 2(2) |
| Missing two vertebrae and a pair of ribs | 7(1) | 0(0) | 7(2) | 0(0) | 8(1) | 2(1) | 7(2) |
| Hemivertebra | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 1(1) |
| Total malformed | 8(2) | 0(0) | 10(4) | 8(6)[a] | 8(1) | 3(2) | 11(6)[c] |

[a] Values differ significantly from the control value ($P<0.05$) using the Wilcoxon test as modified by Haseman and Hoel (1974).

[b] This malformation was observed only in fetuses with short tail.

[c] Value differs significantly from the control value ($P = 0.06$) using the Wilcoxon test as modified by Haseman and Hoel (1974).

acrylonitrile, there was no significant single major malformation observed; however, when malformations were considered collectively, there was a significant (P = 0.06) but slight increase in the 80 ppm (174 mg/$m^3$) exposure group. At the high dose level in the gavage-treated animals, there was a significant increase in acaudate or short-tailed fetuses. The majority of other abnormalities including short trunk, anteriorly displaced ovaries, missing ribs, and imperforate anus were observed in the acaudate and short-tailed fetuses whether these animals were from the control or experimental group. The only anomaly that occurred solely in treated animals was a right-sided aortic arch, which appeared in one fetus from the 25 mg/kg/day group and one fetus from the 65 mg/kg/day group. This anomaly had never been noted in over 1000 litters examined by this laboratory.

The authors concluded that the malformations that occurred in the 65 mg/kg/day group were caused by effects of acrylonitrile directly on the fetus, and not by effects on the dam. This conclusion was based on historical data indicating that mild stress, as caused by acrylonitrile treatment, did not lead to these types of malformations and on the lack of correlation between gross signs of toxicity in the dams (e.g., poor weight gain, and decrease in food consumption) and litters with malformed fetuses. There also appeared to be a dose response relationship because the 25 mg/kg/day group and the 80 ppm (174 mg/$m^3$) exposure group also showed increased incidences of malformed fetuses. This was only suggestive, however, since these increases were not statistically significant when compared with control animals.

Acrylonitrile was also evaluated in chick embryos (Kankaanpaa _et_ _al_., 1979). Acrylonitrile was injected into either the air space or yolk sac of 3-day old White Leghorn eggs. The acrylonitrile was dissolved in saline at concentrations of 0.001, 0.01, 0.1, 1.0, and 10 µmol. A total of 25 µl was used for injection. The only observable adverse effect of treatment was embryo toxicity (dead embryos and empty eggs) of approximately 100% and 44% at the two highest concentrations when acrylonitrile had been injected into the air space. When acrylonitrile was injected into the yolk sac, only the 10 µmol dose was toxic (60%). There was no evidence of a teratogenic effect of acrylonitrile treatment in this study.

Beliles et al. (1980) performed an extensive three-generation reproductive study on Charles River rats that ingested either 100 or 500 ppm acrylonitrile in drinking water. The rats were initially exposed following weaning, and mated 100 days after the start of the experiment. Two litters were produced from each female and members of the second litter were used to produce the next generation (rats from the first litter were used only when an insufficient number of pups was available from the second litter). The parents of the first generation showed some adverse effects of treatment in the 500 ppm group, with food and water consumption and body weights significantly lower than those of control animals (other generations were not monitored for these parameters). Reproductive toxicity was observed in the two matings of the first generation, with an increased number of deaths during the lactation period among pups of animals that had been treated at the 500 ppm level. These deaths may have been a result of acrylonitrile's effect on the dams, since pups fostered by untreated dams had normal survival. In the other generations, reproductive capacity and pup survival were within the normal range. The only adverse effect observed in pups that survived treatment was a decrease in body weight in the 500 ppm group (Table 13-16).

Poor weight gain in the pups may have been caused by poor lactation in the dams due to the decreased water consumption. Ten weanlings of each sex from the control and high dose groups of the F3b litter were sacrificed and examined for

Table 13-16. Pup Weight on Days 4 and 21 of Lactation
(Beliles *et al.*, 1980)

| Generation | Dose Level (ppm) | Mean Pup Weight (g) Day 4 | Mean Pup Weight (g) Day 21 |
|---|---|---|---|
| F1a | 0 | 11 | 42 |
| | 100 | 10 | 40 |
| | 500 | 9[a] | 28[a] |
| F1b | 0 | 10 | 38 |
| | 100 | 9 | 35 |
| | 500 | 10 | 34[a] |
| F2a | 0 | 11 | 39 |
| | 100 | 10 | 39 |
| | 500 | 9[a] | 30[a] |
| F2b | 0 | 11 | 51 |
| | 100 | 10 | 46 |
| | 500 | 9 | 30[a] |
| F3a | 0 | 10 | 43 |
| | 100 | 9 | 43 |
| | 500 | 8[a] | 30[a] |
| F3b | 0 | 10 | 49 |
| | 100 | 10 | 46 |
| | 500 | 8[a] | 32[a] |

[a] $p<0.05$ using Students t-test.

histopathological changes. No adverse findings were noted in the tissues listed in Table 13-17. It appeared that acrylonitrile had little direct effect on the development of the embryo and pup up to the time of weaning.

Table 13-17. Tissues Examined for Histopathologic Changes in the F3b Litter (Beliles et al., 1980)

Adrenal gland
Aorta
Bladder, urinary
Bone
Bone marrow
Cerebellum
Cerebrum
Colon
Esophagus
Eye
Heart
Ileum
Jejunum
Kidney
Liver
Lung
Lymph node
Mammary gland
Muscle, skeletal
Nerve, sciatic
Ovary
Pancreas
Parathyroid
Pituitary gland
Prostate
Salivary gland
Seminal vesicle
Skin
Spinal cord
Spleen
Stomach
Testis
Thymus
Thyroid
Tongue
Trachea

### 13.3.1. Summary and Conclusions

Acrylonitrile adversely affected pup survival following exposure of pregnant rats and, in one study, produced teratogenic events. In a three-generation study in which rats were exposed to 500 ppm acrylonitrile in the drinking water, there was reduced pup survival in the first generation (Beliles et al., 1980). This was a maternal effect inasmuch as fostering the pups on untreated dams eliminated the poor survival. Reproductive capacity was unchanged in the other generations, and the offspring showed no adverse effects on development. Similarly, rats exposed by inhalation to 40 or 80 ppm of acrylonitrile for 6 hours/day on days 6 to 15 of gestation had no statistically significant changes in reproductive success or fetal development (Murray et al., 1978). Only the pups of rats administered acrylonitrile per os (65 mg/kg) for days 6 to 15 of gestation had an increase in malformations (Murray et al., 1976). This increase was in total malformations, with no statistically significant increase occurring in any single malformation. It was concluded that these fetal abnormalities were the result of acrylonitrile and not the result of toxicity in the dams. Although several studies have been conducted to evaluate the ability of acrylonitrile to cause adverse teratogenic, embryotoxic, and reproductive effects, the limitations of the available data do not allow for a full assessment of these effects.

## 13.4 MUTAGENICITY

The objective of this mutagenicity evaluation is to determine whether acrylonitrile has the potential to cause mutations in humans. This qualitative assessment is based on available information derived from several tests that measure different types of genetic damage (e.g., gene mutation, chromosome damage, DNA strand breakage). These tests include assays in bacteria, *Drosophila*, plants, cultured mammalian cells, and whole rodents. The results of these studies on acrylonitrile are discussed below, and each study is summarized in Table 13-18.

### 13.4.1 Gene Mutation Studies

### 13.4.1.1 Bacteria

Acrylonitrile has been shown to cause point mutations in bacteria. Venitt *et al*. (1977) evaluated the mutagenicity of acrylonitrile (purity > 99%, impurities not reported) in a plate incorporation assay with the tryptophan-dependent *Escherichia coli* strains WP2 (repair-proficient), WP2 *uvrA* (lacks excision repair), WP2 *uvrApolA* (lacks excision repair and DNA polymerase I), and WP2 *lexA* (deficient in an error-prone pathway). In the absence of rat liver enzyme activation, acrylonitrile at concentrations of 0, 75 umoles (4000 ug)/plate, and 150 umoles (8000 ug)/plate produced a weak dose-related mutagenic response in all strains except WP2 *lexA*. It should be pointed out that acrylonitrile is volatile and that no precautions to prevent evaporation were reported. If evaporation occurred under the test conditions, the responses observed may represent an underestimation of the mutagenicity of acrylonitrile in this system. The magnitude of the responses are also difficult to interpret because no positive control data were given, and it is therefore unclear how well the test system was responding to mutagen treatment.

The responses reported were as follows: WP2 was slightly more sensitive to the mutagenic effect of acrylonitrile than the other tester strains; a fourfold increase in the spontaneous level was produced by 150 umole/plate compared with a threefold increase for WP2 urvA and a twofold increase for WP2 uvrApolA. The authors reported that doses above 150 umole per plate caused a decline in the mutagenic response, which was explained by increasing toxicity (reduction in bacterial lawn observed).

Because of the toxicity of acrylonitrile in the plate test, Venitt et al. (1977) performed a fluctuation test, which is reputed to be a sensitive assay for detecting low levels of mutagens, at concentrations that ranged from 4 x $10^{-4}$ M to 2 x $10^{-3}$ M. The results of the fluctuation tests confirmed the mutagenicity detected in WP2, WP2 uvrA (results based on one experiment; data not presented), and WP2 uvrApolA in the plate test. In contrast to the results in the plate tests, WP2 uvrApolA was more sensitive to the mutagenic effects of acrylonitrile than WP2 in the fluctuation test; the responses of WP2 and WP2 uvrA were reported as similar. Acrylonitrile again tested negative in WP2 lexA. The lack of a mutagenic effect in WP2 lexA suggests that acrylonitrile may produce mutations by misrepair of DNA damage, which is believed to be associated with the generation of DNA strand breaks (Green and Muriel, 1976). When R-factor plasmid pKM101 was transferred to WP2 (strain designated WP2P) to increase the sensitivity of this strain, WP2P was reported to be more sensitive to the mutagenic effects of acrylonitrile than WP2. A dose-related increase in mutagenic activity was observed at a concentration range as low as 4 umoles (212 ug)/plate to 40 umoles (2122 ug)/plate in the plate test. These results in E. coli strain WP2P further support the notion that acrylonitrile is producing mutations via misrepair of DNA damage because Salmonella typhimurium strains containing the pKM101 plasmid have been shown to be more sensitive to the mutagenic effects of chemicals whose mechanism of action depends on error-prone DNA repair (McCann et al., 1975).

Venitt et al. (1977) indicated that acrylonitrile in the presence of an exogenous activation system (S-9 mix prepared from livers of Aroclor 1254-induced rats) did not cause an increase of revertants of E. coli; thus, according to the authors, acrylonitrile is primarily detected as a direct-acting mutagen in these bacteria. Because data were not presented in this report, however, it is uncertain whether Venitt et al. included an activation-dependent control mutagen in the study to demonstrate that the S-9 fraction used was functional. However, Dr. D.E. Levin of Bruce Ames' laboratory also found that acrylonitrile caused a dose-related mutagenic response [10-fold increase above the background number of revertants at 800 ug (1 ul)/plate; personal communication, December 1982] in the absence of rodent liver S-9 mix when evaluated in the Salmonella plate test using a recently developed tester strain called TA102. This new S. typhimurium strain is much like the E. coli WP2 strains in that TA102 contains a A:T base-pair at the site of mutation (Levin et al., 1982). This is in contrast to all previously developed Salmonella tester strains (e.g., TA1535, TA98, TA100, etc.) that have been used in mutagen evaluation; they detect mutagens that damage G:C base-pairs at the site of reversion. The results from the "G:C" strains are discussed below.

Venitt et al. (1977) reported that acrylonitrile was not detected as mutagenic in Salmonella tester strains hisD3052, TA1535, TA100, TA1538, and TA98 using either plate incorporation assays or fluctuation tests. Although mutagenic activity was reported to be detected in strain hisG46 using a fluctuation test, the results were reported as "erratic and statistically nonsignificant." Because no protocol was presented, it is not known whether mammalian liver activation was employed in the Salmonella assays or if precautions to prevent evaporation were taken. In addition, no data were presented to support the negative results reported in Salmonella. Therefore, Venitt's conclusion of nonmutagenicity in the Salmonella strains cannot be evaluated.

McMahon et al. (1979) reported that acrylonitrile (purity not reported) was mutagenic in both E. coli and S. typhimurium when screened in a qualitative gradient plate assay. However, they did not indicate if acrylonitrile required metabolic activation in these strains, the concentration(s) at which activity was observed, and which bacterial strains were reverted. Therefore, the conclusions cannot be evaluated.

Milvy and Wolff (1977) reported positive results for acrylonitrile (99% purity, impurities not reported) when tested as a vapor in a modified Salmonella/microsome assay using standard tester strains. The mutagenic effects of acrylonitrile were observed only in the presence of metabolic activation (S-9 mix prepared from livers of Aroclor 1254-induced male Swiss-Webster mice); data from tests in the absence of S-9 activation were not presented. Acrylonitrile caused about a doubling in background revertants for tester strain TA1535 when cells were exposed to vapor or when cells were preincubated. Although the data suggests a positive result, the interpretation of this study is difficult because of deficiencies in the reporting of the data: positive control data were not reported and the revertants for each plate were not reported (thus the variation in revertant counts is not known). In addition, Venitt (1978) pointed out errors in calculation found in this report. The studies by DeMeester and coworkers (discussed in the following paragraph), however, provide more convincing evidence for the mutagenicity of acrylonitrile in S. typhimurium (containing

G:C base-pairs at the site of reversion).

DeMeester _et al_. (1978) and other investigators (Venitt _et al_., 1977) have noted that acrylonitrile is not detected as mutagenic in _Salmonella_ tester strains (which have G:C base-pairs at the site of mutation) when assayed in a "classical" plate incorporation assay with or without liver activation.* But DeMeester _et al_. (1978) were able to detect mutagenic activity in _Salmonella_ when the bacteria were exposed to acrylonitrile as a vapor in a desiccator only in the presence of a S-9 mix. The requirement of mammalian liver activation for a positive response in this case and the positive results with the new _Salmonella_ tester strain TA102 (Levin, personal communication) and _E. coli_ WP2 (Venitt _et al_., 1977) in the absence of exogenous liver activation may be ascribed to the ability of acrylonitrile to react directly with the A:T sites in the DNA to form an adduct that reverts _E. coli_ WP2 and _Salmonella_ TA102 but requires biotransformation to revert the _Salmonella_ "G:C" strains.

In the study by DeMeester _et al_. (1978), 0.15 liters of gaseous acrylonitrile (99% purity, impurities not reported) was injected into a desiccator, and the bacteria were exposed for one hour. Using a gas chromatograph, the concentration of acrylonitrile was determined to be about 0.2% in the atmosphere. The concentration of acrylonitrile on the test plates was also measured by freezing the plate agar and analyzing the concentration of acrylonitrile by gas chromatography. This was found to be about 200 ug/plate. The cellular toxicity of 0.15 liters of gaseous acrylonitrile was reported as weak (cell survival 80% to 100%) after a one-hour exposure, and the toxicity was reported to be high when the exposure was either 0.15 liters of gaseous acrylonitrile for 2 hours or 0.24 liters for one hour (the method used to measure toxicity was not described). Reversion to histidine prototrophy was reported for the base-pair substitution-sensitive strains TA1535, TA1530, TA100, and TA1950 and for the frameshift-sensitive strains TA1538, TA1978, and TA98 in the presence of microsomal activation (Aroclor 1254-induced rats, 300 ul S-9/ml mix). Acrylonitrile had the most pronounced effect on TA1530, TA1535, and TA1950, with TA1530 exhibiting the highest number of revertants over the spontaneous number (up to approximately a 15-fold increase over the negative control). Activity in base-pair substitution-sensitive strains would be consistent with the properties of an alkylating agent. Strains TA98, TA1978, and TA100 were only weakly reverted (approximately a twofold or less increase over spontaneous level). Negative results were found with the strains TA1975, TA1532, TA1537, and hisG46. A fluctuation test was conducted to confirm the sensitivity of TA1530 to the mutagenic effects of acrylonitrile. Mutagenic activity was detected at a concentration as low as 2.5 ug/ml ($P < 0.001$). Although DeMeester and coworkers evaluated only one dose and thus do not demonstrate a dose-related mutagenic response, their studies do indicate a clear increase in the number of revertants over the background number, and the increase appears to be reproducible.

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*In the plate incorporation test, the chemical is mixed with the bacteria, S-9 mix, and then melted top agar is immediately added. This mixture is poured on petri plates, which are then incubated 2 to 3 days at 37°C.

Available studies show acrylonitrile to be a mutagen in bacteria. Although the data suggest its mutagenicity to be weak to moderate, there is not yet adequate basis for categorizing the potency of acrylonitrile. It is difficult to quantify the mutagenic activity of acrylonitrile from the studies of DeMeester et al. (1978) or Venitt et al. (1977), because no concurrent positive control chemicals were evaluated, and in the DeMeester et al. (1978) study only one dose was tested. (The data from Dr. Levin is not yet available.) Because of this, Monsanto (Kier, 1982a) conducted a modified liquid suspension assay using standard tester strains of *S. typhimurium* exposed to several concentrations of acrylonitrile and concurrently tested several standard mutagens (4-nitroquinoline-N-oxide, 2-acetylaminofluorene, 2-aminoanthracene, benzo[a]pyrene) to determine the relative activity of acrylonitrile to these known mutagens. In this test, the bacteria, chemical, and S-9 mix (Aroclor 1254-induced rat liver) were mixed and incubated for two hours at 30°C. After a 2-hour exposure to concentrations of acrylonitrile that ranged from 20 ug/ml to 20,000 ug/ml, the cells were washed, mixed with top agar, plated in petri dishes, and incubated at 37°C for 2 days before scoring. Viability was not quantitatively determined in these experiments (only the observation of bacterial lawn growth or presence of small colonies). It was concluded that acrylonitrile was weakly mutagenic in tester strain TA100 with S-9 activation but not in TA98 and TA1535. No increased responses were observed without S-9 mix. In TA100, average revertant counts at the highest dose (6000 ug/ml) tested was about 1.5-fold over the solvent control. Therefore, under the liquid suspension treatment conditions used by Monsanto, acrylonitrile caused, at most, a marginal increase in the number of revertants. However, these studies alone do not allow for a quantitation of the mutagenic activity of acrylonitrile.

The bacterial studies by DeMeester *et al*. (1978) and Kier (1982a) indicate that acrylonitrile was detected as mutagenic in the *Salmonella* "G:C" strains only when metabolized *in vitro* by homogenates of mammalian liver. In addition to acrylonitrile being activated by *in vitro* S-9 systems, Lambotte-Vandepaer *et al*. (1980) examined the ability of the whole mammal to metabolize acrylonitrile by testing urine from rodents exposed to acrylonitrile in *Salmonella*. These authors reported that urine (0.1 ml per plate) from both rats (adult male Wistar) and mice (NMRI) treated with a single intraperitoneal dose of acrylonitrile (30 mg/kg; purity 99%, impurities not reported) was mutagenic in Salmonella tester strain TA1530 (9-fold increase in the spontaneous level of revertants when exposed to mouse urine and 13-fold increase for exposure to rat urine). S-9 mix (from Aroclor-1254 treated mice) added to the test plates caused a fivefold reduction in the mutagenicity of urine from acrylonitrile-treated rats. Only a slight reduction (1.3-fold) was seen with the urine of acrylonitrile-treated mice. When animals were pretreated with phenobarbital and exposed to acrylonitrile, the urine from rats produced no detectable mutagenicity and the mutagenicity of urine from mice was reduced. The addition of B-glucuronidase to the test plates only slightly enhanced (approximately 1.4-fold) the mutagenic effects of urine collected from acrylonitrile-treated rats and mice (B-glucuronidase is added to cleave possible conjugates). However, B-glucuronidase had a marked effect on the mutagenicity of urine from rats treated with phenobarbital and exposed to acrylonitrile (an eightfold increase in mutagenic activity observed). The authors indicated that XAD-2 resin concentrates of urine from all treatment groups were not detected as mutagenic (data not presented), suggesting that the mutagenic metabolite(s) was very hydrophilic. Therefore, the urine from acrylonitrile-exposed rats and mice was mutagenic in Salmonella, and glucuronoconjugation appeared to play a minor role in the deactivation of the acrylonitrile derivative(s) (except in the case of phenobarbital/acrylonitrile-treated rats, where a greater effect was found).

Conner et al. (1979) also examined whole mammal activation of acrylonitrile and found that bile obtained from rats treated with acrylonitrile (intraperitoneal injection of 45 mg/kg body weight) was not mutagenic in Salmonella TA1535. However, these negative findings are questionable because data were not presented in the report and several known mutagens (e.g., dimethylnitrosamine, 3-methylcholanthrene), which require metabolic activation, and the direct-acting mutagen methyl methanesulfonate, were also negative in this study.

Studies using Salmonella tester strains that contain G:C base-pairs at the site of mutation indicate that metabolic activation of acrylonitrile produces a mutagenic form(s). A potential reactive metabolite of acrylonitrile is an epoxide. Monsanto (Kier, 1982b) evaluated the acrylonitrile epoxide 2,3-epoxypropionitrile (purity 97.4%), in a modified liquid suspension Salmonella assay (described previously) and found it to be mutagenic in the standard tester strains TA100, TA1535, and TA98 in the absence or presence of Aroclor-1254 induced rat S-9 mix. This epoxide was most active in the base-pair substitution-sensitive strains. For example, in TA100 with S-9 mix, a fourfold increase in revertant counts over background counts was seen at 400 ug/ml. This epoxide does not appear to be a strong-acting mutagen under the liquid suspension treatment conditions used in this study when compared to the positive control mutagens 4-nitroquinoline-N-oxide, 2-acetylaminofluorene, 2-aminoanthracene, and benzo[a]pyrene.

### 13.4.1.2 Drosophila

Although acrylonitrile has been shown to cause point mutations in bacteria, its mutagenicity in a eucaryotic organism has not been adequately examined. The only available eucaryotic gene mutation report is a study by Benes and Sram (1969) in which the occurrence of sex-linked recessive lethal mutations in Drosophila melanogaster was examined. A 0.1% solution of acrylonitrile (purity not reported) was injected into the abdomen of male flies. (The authors do not give the percent survival but indicate that chemical concentrations given to male flies were the highest possible, being slightly below a lethal or sterile dose.) Although the authors report the results as nonsignificant, the data can be considered as suggestive of an increase in the mutation frequency by a factor of three over that of the spontaneous frequency. Several deficiencies were found in this report.

1. A small sample size of flies was tested (1297 chromosomes). In order to preclude a doubling in mutation frequency, 7000 chromosomes would have to be tested (Lee _et al_., in press).
2. No information was given on whether clustering of mutations occurred.
3. The purity of the test material was not described.
4. Post-mating days were not given.
5. No information was reported for concurrent positive or historical negative controls.

Acrylonitrile has, therefore, not been properly evaluated in this system, and retesting is necessary to permit a final judgement on the mutagenic activity of acrylonitrile in _Drosophila_.

### 13.4.2 Chromosomal Aberration Studies

The ability of acrylonitrile to induce chromosomal aberrations _in vivo_ has been investigated in both rats and mice. Acrylonitrile was not detected as clastogenic in two independent bone marrow assays (Rabello-Gay and Ahmed, 1980, Leonard _et al_., 1981). Rabello-Gay and Ahmed (1980) tested acrylonitrile (purity reported as 99.5%, impurities not identified) for chromosomal effects in mice (Swiss albino) and rats (Sprague-Dawley). No significant increase (at a confidence level of 0.05) in the incidence of chromosomal aberrations (gaps, breaks, fragments, Robertsonian translocations) was found in mouse bone marrow cells. Male mice were given acrylonitrile by gavage for 4, 15, and 30 days each at doses of 7, 14, and 21 mg/kg/day, and at least 1200 cells were examined per treatment group. In a second experiment, mice received 10, 15, and 20 mg/kg/day, for the same time intervals. The toxicity of 7, 14, and 20 mg/kg/day given orally was reported to correspond to 0.25, 0.50, and 0.75 of the $LD_{50}$, respectively. Mortality for the acrylonitrile-treated animals was reported (3 of the 72 animals died). The chemical also was found to be negative when rats were exposed orally to 16 doses at 40 mg/kg/day (reported to represent one-half of the $LD_{50}$). Because current evidence (Abreu and Ahmed, 1980, and Guengerich et al., 1981) indicates that acrylonitrile can be biotransformed to cyanide, these authors also evaluated potassium cyanide (KCN) and found no increase of chromosomal aberrations in the bone marrow cells of rats treated with 16 oral doses of KCN (5 mg/kg/day, 0.5 $LD_{50}$). It should be noted that concurrent or historical positive controls were not reported in the study by Rabello-Gay and Ahmed (1980).

Leonard et al. (1981) evaluated the clastogenicity of acrylonitrile (purity not given) in mouse bone marrow cells in vivo. These authors conducted both chromosomal aberration and micronucleus analyses. The percentage of chromosomal aberrations or micronuclei did not differ between acrylonitrile-treated animals and negative control animals. Male mice (NMRI) were injected intraperitoneally with a single acute dose of 20 or 30 mg acrylonitrile/kg of body weight. The authors indicated that 30 mg/kg was the maximum dose that allows survival of mice for several weeks. For chromosomal aberration analysis (gaps, breaks, fragments, rearrangements), 200 cells (4 animals and 50 cells/animal) were examined 6, 18, 24, 48, and 72 hours after each treatment. The background control frequency was 0.5% cells with chromosomal anomalies (1 gap/200 cells), and the acrylonitrile-treated animals did not exceed 1.5% cells with chromosomal anomalies (3 gaps/200 cells). It should be noted that gaps are not regarded as true chromosome aberrations at this time. For micronucleus analysis, polychromatic erythrocytes were sampled 24, 48, or 72 hours after initial treatment. The background frequency was 1.8% cells with micronuclei, and acrylonitrile-treated cells did not exceed 2.5% with micronuclei. No dose-related effects were seen in these studies.

Leonard et al. (1981) also conducted a mouse-dominant lethal assay with acrylonitrile. This test evaluates the DNA-damaging effects of chemicals on germinal tissue. Male mice (NMRI) were given a single injection of 30 mg of acrylonitrile/kg of body weight followed by a weekly mating (1 to 5 weeks). The authors concluded that there was no difference in the percent of postimplantation loss between the background control group and the acrylonitrile-treated groups. The findings in this test, however, are considered inconclusive because of the high frequency of dominant lethals observed in the negative control group (33.6% postimplantation loss and only 41% pregnant females).

Thiess and Fleig (1978) conducted a cytogenetic analysis of lymphocytes from 18 workers who had been exposed to acrylonitrile. The workers had been exposed for an average of 15.3 years and were compared with 18 age-matched workers who had no known exposure to acrylonitrile or any other compounds that were suspected of causing chromosomal damage. Although no information is available concerning the exact levels of exposure in the past, atmospheric monitoring data betweeen 1963 and 1974 indicated a typical exposure level of 5 ppm for acrylonitrile, and atmospheric monitoring data between 1975 and 1977 indicated an average exposure level of 1.5 ppm. The authors point out that individual workers may have been exposed to higher concentrations during specific operations. Also, the workers could have been exposed on the job to styrene, butadiene, ethylbenzene, butylacrylate, and diphyl (a mixture of diphenyl and diphenyl oxide ether). For each subject, 100 metaphases were examined. Types of chromosomal aberrations were not classified in the report except for gaps and isogaps. Frequencies of chromosomal aberrations in the exposed group were 1.8 ± 1.3% (excluding gaps) and 5.5 ± 2.5% (including gaps and isogaps), while chromosome aberrations in the control group were 2.0 ± 1.6% (excluding gaps) and 5.1 ± 2.4% (including gaps and isogaps). Therefore, chromosomal damage was not detected in these workers.

Loveless (1951) and Kihlman (1961) reported negative findings for chromosomal effects in plants (*Vicia faba*); however, data were not reported in these articles, and details of the protocol were not presented. Thus, the negative conclusions of the authors cannot be evaluated.

### 13.4.3 Other Tests Indicative of Genetic Damage

Other tests have been conducted which do not measure mutation per se but may indicate that acrylonitrile has the potential to cause mutations.

Chemical-adduct formation in DNA is a critical event in certain types of mutagenesis. DNA binding studies have been conducted using radiolabeled acrylonitrile to determine its ability to react with DNA. Guengerich et al. (1981) provided evidence that acrylonitrile can covalently bind with DNA *in vitro*.* These authors found a low level of $^{14}C$-labeled acrylonitrile binding to calf thymus DNA when a metabolizing system (uninduced-rat liver microsomes plus NADPH) was included in the reaction mixture. A twofold increase in DNA binding over the response observed with uninduced rat microsomes was seen when liver microsomes were derived from rats pretreated with phenobarbital, an inducer of cytochrome P-450. There was a much higher level of binding to microsomal protein than to DNA (protein to DNA ratio was approximately 20 to 1). It should be pointed out that several known mutagens (e.g., ethylene oxide, methyl methanesulfonate) have been shown to alkylate protein to a greater extent than DNA (Ehrenberg and Ostermar-Golkar, 1980. Ehrenberg, 1979). When Guengerich and coworkers (1981) used rat brain or human liver microsomes plus NADPH, the binding to DNA was much lower than when rat liver microsomes were used for metabolic activation. Apparently, the *in vitro* rat brain microsomes do not metabolize acrylonitrile as effectively under the conditions tested. Because information was not given concerning the freshness of the human autopsy liver species used to prepare the microsomes, the ages of the human subjects that the livers came from, and the cause of death of the human subjects, it is difficult to conclude that human liver microsomes do not effectively metabolize acrylonitrile. These authors also showed that the rat liver microsomes (or a reconstituted cytochrome P-450 system) metabolize acrylonitrile to 2,3-epoxypropionitrile, an epoxide. This epoxide was shown to be stable and also to bind calf thymus DNA and protein.

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*Acrylonitrile has been shown to react with certain minor tRNA nucleosides, and, at a slower rate, ribothymidine and thymidine (Ofengard, 1967 and 1971.

Glutathione S-transferase appeared to play a role in the deactivation of acrylonitrile (and/or its metabolites) in these *in vitro* studies by Guengerich *et al*. (1981). A rat liver cytosol preparation of glutathione conjugated 2,3-epoxypropionitrile at a greater rate than it conjugated acrylonitrile. Human liver and rat brain cytosol preparations reacted with 2,3-epoxypropionitrile at a much lower rate than did rat liver and not at a detectable rate with acrylonitrile. Therefore, if acrylonitrile was activated to 2,3-epoxypropionitrile and reached the brain via blood circulation, this epoxide may not be effectively inactivated. It should be cautioned, however, that these are *in vitro* tests, and studies using radiolabeled acrylonitrile of high specific activity are needed in whole mammals to elucidate the events *in vivo*. Also, because of the low level of DNA binding detected in these *in* vitro studies, it would be more convincing and informative if specific DNA adducts were characterized instead of simply measuring covalent DNA binding. These results, however, are consistent with the results obtained from mutagenicity studies using the Salmonella "G:C" strains and in Chinese hamster ovary cells (to be discussed) in that metabolic activation was required for a detectable response.

Monsanto has sponsored preliminary experiments to study alkylation of DNA in various tissues of rats (Dr. Paul Wright, personal communication). During an EPA Science Advisory Board Meeting (August 2, 1982), Monsanto indicated that no binding of acrylonitrile to DNA was detected in these experiments. Although Monsanto made the preliminary data available to EPA, without details of the protocol and additional data, a definitive judgement cannot be made regarding these whole mammal tests. It appears, however, that the specific activity of the radiolabel was too low to preclude the possibility of DNA binding in these studies. In addition, only a nontoxic dose (approximately 10 mg/kg) was evaluated. Therefore, further studies are needed using radiolabeled acrylonitrile with a higher specific activity and using several doses (including a toxic dose).

If a chemical reacts with DNA, it may ultimately cause genetic damage. Parent and Casto (1979) reported that acrylonitrile caused single strand DNA breaks in primary Syrian Golden hamster embryo cells in vitro as detected by alkaline sucrose gradient sedimentation. When cells were treated for 18 hours at concentrations of 200 or 400 ug of acrylonitrile/ml, shifts in sedimentation patterns were observed. This effect did not occur at 50 ug/ml or 100 ug/ml. The authors stated that these shifts are comparable to those produced by known carcinogens and are not seen with noncarcinogenic chemicals. They concluded that the results are suggestive of carcinogenicity. However, because the toxicity of acrylonitrile at the concentrations tested was not given, it is uncertain whether the DNA damage observed at 200 or 400 ug/ml is a reflection of premutational damage or of nonspecific toxicity. It should be noted that these data were generated in the absence of an exogenous metabolic activation system.

Dr. G. Williams evaluated the potential of acrylonitrile to damage DNA by measuring DNA repair synthesis in a rat hepatocyte primary culture (personal communication, 1982). A major advantage of the intact hepatocyte test system is that it provides a better approximation of the activation/deactivation of a chemical substance as it would occur in the rat liver in vivo than does an exogenous S-9 system. Dr. Williams indicated that acrylonitrile was negative in this system; however, he emphasized that the test agent was intensely toxic to the hepatocytes. He found that the hepatocytes biotransform acrylonitrile to cyanide, which is very toxic to the liver. Thus, these negative findings do not necessarily indicate the inability of acrylonitrile to damage DNA. This conclusion is supported by other studies by Dr. S. Ved Brat and Dr. G. Williams (unpublished, 1982) that have shown a significant increase in sister chromatid exchange (SCE) formation in Chinese hamster ovary (CHO) cells when metabolic activation is mediated by rat hepatocytes.

S. Ved Brat and G. Williams (1982, unpublished) studied the induction of SCE in CHO cells by acrylonitrile. The evaluation of SCE formation, which is thought to involve DNA breakage, is a very sensitive method that can detect genotoxic effects at lower concentrations than those needed to detect an increase in chromosome aberrations (Wolff, 1977; Latt, 1974). In two separate experiments, exposure to acrylonitrile at five concentrations, from $10^{-7}$M up to $10^{-4}$M, did not produce SCE in CHO cells without an exogenous metabolic activation system. In contrast, acrylonitrile caused a progressive increase in the SCE incidence when CHO cells were cocultured with rat hepatocytes for metabolic activation. The highest response (approximately twofold increase over background levels) was observed at $10^{-4}$M acrylonitrile. It should be pointed out that coculturing hepatocytes with the CHO cells resulted in an elevated background level of SCE in CHO (twice that normally observed). Nevertheless, in two separate experiments a dose-related response was demonstrated. These results indicate that the rat liver cells can mediate the activation of acrylonitrile and that the reactive metabolite(s) apparently reaches the CHO cells where it causes genetic damage.

### 13.4.4 Summary and Conclusions

Acrylonitrile has been evaluated for its ability to cause gene mutations in bacteria and *Drosophila* and for its ability to cause chromosome damage in plants and rodents. Acrylonitrile-exposed workers have also been screened for induced chromosome damage. Other endpoints indicative of mutagenicity have been examined, such as DNA repair synthesis and sister chromatid exchange in cultured mammalian cells and DNA binding *in vitro*.

There is evidence that acrylonitrile can cause point mutations in both *Salmonella typhimurium* and *Escherichia coli*. When A:T base-pairs (i.e., *Salmonella* TA102 and *E. coli* WP2) are at the site of reversion, acrylonitrile is mutagenic without an exogenous liver activation system; however, when acrylonitrile is tested in *Salmonella* tester strains with G:C base-pairs at the site of mutation, the presence of an S-9 activation system is required to detect mutagenic activity. The epoxide of acrylonitrile, 2,3-epoxypropionitrile (a potential metabolite), has been shown to be mutagenic in *Salmonella*. Because acrylonitrile (and the metabolite) can cause point mutations in bacteria, it may cause point/gene mutations in other organisms as well. Results of acrylonitrile from a sex-linked recessive lethal test in *Drosophila* provide some support for this expectation in that the data are considered suggestive of an increase in mutation frequency over that of the spontaneous frequency by a factor of three. Further testing, however, is needed in other eucaryotic organisms to confirm the ability of acrylonitrile to cause point/gene mutations.

Acrylonitrile has not been shown to cause chromosome aberrations. Studies in plants have been reported to be negative. In rats and mice, acrylonitrile (when evaluated up to toxic doses) was not found to be clastogenic in bone marrow cells. In addition, no apparent chromosomal damage was detected in peripheral blood lymphocytes from workers exposed to acrylonitrile. However, acrylonitrile did induce an increase in sister chromatid exchange (SCE) formation in Chinese hamster ovary (CHO) cells *in vitro* when metabolic activation was mediated by intact rat hepatocytes. The induction of SCE is thought to involve DNA breakage and this effect can be detected at concentrations lower than those needed to detect an increase in chromosome aberrations. (These studies strongly reinforce the need to further examine the ability of acrylonitrile to cause gene mutations in eucaryotic organisms.) Acrylonitrile was negative in a DNA repair test using primary rat hepatocytes. In this test, acrylonitrile was intensely toxic to the liver cells because of the production of cyanide. Thus, the compound cannot be adequately evaluated in the rat hepatocyte-DNA repair test system.

DNA binding studies indicate the potential of acrylonitrile to react with DNA, a critical event in certain types of mutagenesis. A low level of covalent binding of radiolabeled acrylonitrile to calf thymus DNA *in vitro* was detected in the presence of exogenous rat liver microsomal activation. A metabolite of acrylonitrile, 2,3-epoxypropionitrile, also binds calf thymus

DNA *in vitro*. However, additional studies are necessary to determine if DNA binding occurs in intact cells *in vitro* and in whole mammals after acrylonitrile exposure.

The ability of acrylonitrile and/or an active metabolite to reach mammalian gonads and cause germ cell mutations has not been adequately studied. The only study available for review was a mouse dominant lethal assay. Although a significant increase in dominant lethals was not detected, the high background of dominant lethals in this study precludes interpreting that acrylonitrile is nonmutagenic in this test.

In conclusion, the weight-of-evidence indicates that acrylonitrile has the potential to cause genetic damage (as shown by point mutation studies in bacteria and SCE studies in cultural mammalian cells and as suggested by *Drosophila* studies). Some available evidence indicates that conversion of acrylonitrile to a metabolite(s), such as an epoxide, may result in a mutagenic form(s). Thus, acrylonitrile may cause somatic mutations in humans if its pharmacokinetics in humans result in metabolic products that can interact with DNA, as is the case in several test systems. Additional mutagenicity studies are needed, in eucaryotic organisms, to confirm that acrylonitrile is mutagenic. Additional tests using radiolabeled acrylonitrile to measure alkylation of DNA in various tissues of whole mammals are also needed. An assessment of genetic risk with respect to germ cell mutagenicity cannot be made yet because of the lack of appropriate data.

## TABLE 13-18. MUTAGENICITY TESTS OF ACRYLONITRILE

A. GENE MUTATION TESTS: BACTERIA

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comment |
|---|---|---|---|---|---|---|
| Connor et al., 1979 | Body fluid analysis: bile from rats, Salmonella typhimurium as indicator organism; plate test | TA1535 | In vivo male Sprague-Dawley rats | 45 mg/kg acrylonitrile in saline injected i.v. into femoral vein, 200 ul of bile tested/plate<br>Source: Aldrich Chemical Company | Negative | 1. Negative findings are inconclusive.<br>2. One bacterial strain tested.<br>3. Number of animals used not reported.<br>4. Number of revertants not reported.<br>5. Purity of chemical not given. |
| DeMeester et al., 1978 | Salmonella test: plate test, fluctuation assay | TA1530<br>TA1535<br>TA1537<br>TA1538<br>TA98<br>TA100<br>TA1950<br>hisG46<br>TA1978<br>TA1532<br>TA1975 | S-9 mix prepared from livers of styrene, Aroclor 1254, acrylonitrile, methyl-3-cholanthrene, phenobarbital -induced male rats (Wistar); Aroclor 1254- and phenobarbital -induced female mice (NMRI); uninduced male beagle dog | Tested in atmosphere at ± 0.2% for 1 hour (200ug/plate); purity reported as 99% (impurties not described)<br>Source: Aldrich Europe | Positive in the presence of metabolic activation in both frameshift -sensitive and base-pair substitution sensitive strains, with most pronounced effects in base-pair substitution sensitive strains. Negative in the absence of metabolic activation. | 1. Responses are considered to indicate mutagenic activity because clear increases in revertant colonies over the background frequencies were observed, and these increases were reproducible.<br>2. No concurrent positive controls reported.<br>3. Single dose tested; thus, no dose-response curve.<br>4. Method to determine toxicity not described. |

*Authors' conclusions.

(continued on the following page)

TABLE 13-18. (continued)

A. GENE MUTATION TEST: BACTERIA

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Kier, 1982a Monsanto Report | *Salmonella*/ microsome assay: modified liquid suspension | TA98<br>TA100<br>TA1535 | S-9 mix from livers of Aroclor 1254-induced rats | Acrylonitrile 0.02 mg/ml to 20 mg/ml liquid material diluted in distilled $H_2O$<br>Purity: > 99% | Weak mutagenicity in strain TA100 only after metabolic activation. | 1. Results considered borderline at most.<br>2. A much higher concentration (43 ug/ml) was used for the TA1535 positive chemical, 2-aminoanthracene, than is generally employed (2.5 ug/plate). The response of this control was weak. Therefore, results in tester strain TA1535 are difficult to interpret. |
| Kier, 1982b Monsanto Report | *Salmonella*/ microsome assay: modified liquid suspension test | TA98<br>TA100<br>TA1535 | S-9 mix from livers of Aroclor 1254-induced rats | 2,3-epoxypropionitrile (acrylonitrile epoxide) 0.2 mg/ml to 20 mg/ml diluted in distilled $H_2O$<br>Purity: 97.4% | Mutagenic in strains TA100 and TA1535, TA100 being the most sensitive strain. Mutagenic activity expressed in absence of S-9 mix and a slight increase was found in the presence of S-9 mix. | 1. These data demonstrate the mutagenicity of acrylonitrile epoxide. |

*Authors' conclusions.

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TABLE 13-18. (continued)

A. GENE MUTATION TESTS: BACTERIA

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Lambotte-Vandepaer et al., 1980 | Body fluid analysis: urine from rats and mice, Salmonella typhimurium as indicator organism; plate test | TA1530 | In vivo adult male Wistar rats and adult male NMRI mice; in vitro, S-9 mix from livers of Aroclor 1254-induced mice (strain not reported) | 30 mg/kg (purity > 99%, impurities not described); in saline injected i.p.; urine collected 24 hours<br>Source: Aldrich, Europe | Positive | 1. Single dose tested, thus no dose-response curve determined, but the data are still considered to indicate mutagenic activity.<br>2. No concurrent positive control data reported. |
| McMahon et al., 1979 | Reversion in Salmonella typhimurium and Escherichia coli; gradient plate test | | | | Reported as positive for both Salmonella and Escherichia | 1. Positive conclusions cannot be evaluated because of lack of data.<br>2. Purity and source of chemical not given.<br>3. Authors did not indicate if mutagenic activity was dependent on exogenous metabolic activation.<br>4. Bacteria strains, which were reverted, were not given.<br>5. Information on toxicity not reported. |

*Authors' conclusions.

(continued on the following page)

TABLE 13-18. (continued)

A. GENE MUTATION TESTS: BACTERIA

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Milvy and Wolff, 1977 | *Salmonella* test: spot assay, liquid suspension, plate test (in vapor phase) | TA1535<br>TA1538<br>TA1978 | S-9 mix from livers of Aroclor 1254-induced male Swiss-Webster mice | Spot test: 5 and 10 ul; liquid suspension: 5, 10, and 20 ul for 30 min. to 4 hours<br>Source: Aldrich Chemical Company<br>Purity: Reported as 99+% | Liquid suspension and vapor: positive only in the presence of metabolic activation in both frameshift and base-pair substitution sensitive strains; negative results using spot test and testing in the absence of metabolic activation. | 1. Positive conclusions are difficult to interpret because of study design problems and lack of data.<br>2. No clear dose-response observed with vapor exposure.<br>3. The actual revertant counts per plate were not given; thus, the extent of variation is not known. |

(continued on the following page)

*Authors' conclusions.

TABLE 13-18. (continued)

A. GENE MUTATION TESTS: BACTERIA

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Venitt et al., 1977 | Escherichia coli test: plate test, fluctuation assay | WP2<br>WP2 uvrA<br>WP2 uvrApolA<br>WP2 lexA<br>WP2P (pKM101) | S-9 mix from livers of Aroclor 1254-induced CB hooded male rats, 0.5 ml/plate | 0, 75, 150 umole/plate for plate incorporation assay $4 \times 10^{-4}$M to $2 \times 10^{-3}$M for fluctuation assay. Dissolved in DMSO, (purity > 99% as determined by GLC) Source: Kochlight Lab | Weak positive in the absence of metabolic activation for all strains except WP2 lexA | 1. Clear dose-related response.<br>2. No concurrent positive control reported.<br>3. Data in the presence of metabolic activation not reported.<br>4. No precautions to prevent evaporation were reported. |
| | Salmonella test: plate test, fluctuation assay | TA100<br>TA1538<br>TA98<br>TA1535<br>hisG46<br>hisD3052 | | | Reported as negative | 1. Negative conclusion cannot be evaluated because data were not presented and protocol was not described. |

*Authors' conclusions.

(continued on the following page)

Table 13-18. (continued)

B. GENE MUTATION TESTS: DROSOPHILA

| Reference | Test System | Dose | Result<br>Brood I<br>(postmeiotic) | <br>Brood II<br>(meiotic and premeiotic) | <br>Total | Comments |
|---|---|---|---|---|---|---|
| Benes and Sram, 1969 | Sex-linked recessive lethal test in *Drosophila melanogaster* | Injection of a 0.1% solution into abdomen; | 2/572 (0.35%) | 4/725 (0.55%) | 0.46% | 1. Results considered suggestive of threefold increase in mutation frequency.<br>2. Toxicity not given.<br>3. Sufficiently large sample size not analyzed (7000 chromosomes are needed to preclude a doubling in mutation frequency).<br>4. Purity and source of chemical was not given.<br>5. No concurrent positive controls, and information concerning negative controls not given; appears to be historical.<br>6. Post-mating times not given. |
| | | spontaneous frequency | | | 58/41,921 (0.14%) | |

(continued on the following page)

TABLE 13-18. (continued)

C. MAMMALIAN IN VIVO CYTOGENETICS

| Reference | Test System | Strain | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|
| Leonard et al., 1981 | Bone marrow assay (micronucleus and chromosomal aberration analysis) | NMRI male mice | Mice given single i.p. injection of 20 or 30 mg/kg and cells examined 6, 18, 24, 48, and 72 hours later for chromosome analysis and 24, 30, and 48 hours later for micronucleus analysis. | Negative | 1. Aberrations scored were gaps, breaks, and rearrangements. |
|  | Dominant lethal assay | NMRI male mice | Mice given i.p. treatment of 30 mg/kg followed by weekly matings (1 to 5 weeks).<br>Source: Aldrich Europe (purity not given) | Negative | 1. Results considered inconclusive because background control frequency was very high (33.6% postimplantation loss). |
| Rabello-Gay and Ahmed, 1980 | Bone marrow assay | Albino Swiss mice, Sprague-Dawley rats | Mice treated at 7, 14, and 20 mg/kg/day by gavage for 4, 15, and 30 days; rats exposed orally to 16 daily doses of 40 mg/kg/day (purity 99.5%).<br>Source: Aldrich Chemical Company | Negative | 1. Aberrations scored were gaps, isogaps, breaks, fragments, and translocations.<br>2. No concurrent or historical positive controls reported. |
| Thiess and Fleig, 1978 | Examination of lymphocytes from workers with known exposure to acrylonitrile |  | Atmospheric monitoring data indicated a typical exposure level of 5 ppm between 1963 and 1974, and a level of 1.5 ppm between 1975 and 1977. | Negative | 1. Cohort was exposed to acrylonitrile for an average of 15.3 years.<br>2. Aberrations scored included gaps and isogaps; other aberrations scored not defined in the report. |

*Authors' conclusions.

(continued on the following page)

TABLE 13-18. (continued)

D. OTHER TESTS INDICATIVE OF GENETIC DAMAGE

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Guengerich et al., 1981 | DNA adduct formation in vitro-calf thymus DNA | | Rat and human liver microsomes, and purified rat liver P-450 | 1mM $^{14}C$-labeled acrylonitrile (purity > 99%) for 1 hour; $^{14}C$-labeled 2-cyanoethylene oxide, 0.25 mM at different times up to 4 hours | Low level of DNA binding detected with rat liver activation for acrylonitrile, 2-cyanoethylene also binds DNA in vitro. | |
| Parent and Casto, 1979 | Detection of single strand DNA breaks by alkaline sucrose gradient | Primary Syrian Golden hamster embryo cells in vitro | None | Cells incubated for 18 hours at 50, 100, 200, and 400 ug of acrylonitrile/ml (≥ 99% pure); and contains 30-45 ppm 1-hydroxy-4-methoxybenzene 0.3% water, < 0.5% acetonitrile. Source: Aldrich Chemical Company | DNA breakage at 200 and 400 ug/ml but not at 50 or 100 ug/ml. | 1. Toxicity data not given; thus, it is unclear whether the results are due to nonspecific toxicity. |

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*Authors' conclusions.

TABLE 13-18. (continued)

D. OTHER TESTS INDICATIVE OF GENETIC DAMAGE

| Reference | Test System | Strain | Activation System | Concentration | Reported Result* | Comments |
|---|---|---|---|---|---|---|
| Ved Brat and Williams, 1982, Unpublished | Sister chromatid exchange (SCE) in CHO cells coculture _in vitro_ with rat hepatocytes | | Intact rat hepatocytes | Cells were exposed 3 hours to acrylonitrile dissolved in DMSO. Dose range from $10^{-7}$ M to $10^{-3}$M. Purity: Not given | Negative results for CHO cells without hepatocytes.<br>Positive results for CHO with hepatocytes. | 1. Response was dose-related in two separate experiments.<br>2. Background level of SCE was very high. |

| | Concentration (M) | SCE/Cell<br>Experiment 1 | <br>Experiment 2 |
|---|---|---|---|
| Positive Control | DMBA 5 x $10^{-5}$ | 33.5 ± 1.8 | 54.5 ± 2 |
| Acrylonitrile | $10^{-3}$ | ---- | Toxic |
| | $10^{-4}$ | 55.7 ± 3.6 | 45.6 ± 1.5 |
| | $10^{-5}$ | 28.2 ± 1.2 | 36.4 ± 1.0 |
| | $10^{-6}$ | 24.9 ± 1.4 | 30.9 ± 1.9 |
| | $10^{-7}$ | 23.7 ± 1.2 | ---- |
| | DMSO (1%) | 21.7 ± 1.7 | 24.9 ± 1.9 |

*Authors' conclusions

## 13.5 CARCINOGENICITY

The purpose of this section is to provide an evaluation of the likelihood that acrylonitrile is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact, including a potency evaluation in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior, have an important bearing on both the qualitative and quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. This section presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and finally, a summary and conclusions dealing with all of the relevant aspects of carcinogenicity.

### 13.5.1 Animal Studies

Seven studies in which acrylonitrile was administered to rats will be discussed. In four studies (three cancer bioassays and one three-generation reproductive study), the route of administration was via drinking water, one study was via gavage, and two (cancer bioassays) were via inhalation.

#### 13.5.1.1 Drinking Water Studies

##### 13.5.1.1.1 Dow Chemical Company (Quast et al., 1980a)

Quast et al. (1980a) of the Dow Chemical Company performed a 2-year chronic study under the auspices of the Chemical Manufacturers Association. The acrylonitrile used in this study was produced by E.I. du Pont de Nemours and

Company, Inc. Its purity was greater than 99%. In this study 6- to 8-week-old male and female Sprague-Dawley rats (48 animals of each sex at each exposure level and 80 animals of each sex in the control group) were given acrylonitrile in drinking water. For the first 21 days, the concentrations given were 35, 85, and 210 ppm; however, the two higher concentrations were subsequently raised to 100 and 300 ppm. After 9 months of treatment, the animals at the two higher doses showed signs of toxicity as indicated by decreased self-grooming and an unthrifty appearance. There was also a dose-related decrease in food consumption (except in male rats at the lowest exposure level), and concomitant dose-related decrease in water consumption. Using water consumption data and the weight of the rats, the calculated mean amount of acrylonitrile ingested for males was 3.42, 8.53, and 21.18 mg/kg/day, and for females, 4.36, 10.76, and 24.97 mg/kg/day for the 35, 100, and 300 ppm exposure levels, respectively.

Cumulative mortality data for male and female rats at all exposure levels are presented in Tables 13-19 and 13-20. The results indicate that early mortality was observed at all dose levels in females.

On death or at necropsy at 24 months, histopathologic examination was performed on complete sets of tissues from the control and high dose animals and on selected target tissues and tissues with grossly recognized tumorous changes from the other exposure groups.

Statistically significant increases in tumor incidence at multiple sites in male and female rats exposed to acrylonitrile were observed as described in Tables 13-21 and 13-22. Statistically significant increases in the incidence of specific tumor types with respect to individual treatment groups are summarized as follows: central nervous system tumors in males and females in all treatment groups; Zymbal gland tumors in females in all treatment groups and in males in the high-dose group; tumors in the nonglandular portion of the stomach in males

## TABLE 13-19. CUMULATIVE MORTALITY DATA OF MALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE
(Quast _et_ _al_., 1980a)

| Days on test | Control No. Dead (% dead) | 35 ppm No. Dead (% dead) | 100 ppm No. Dead (% dead) | 300 ppm No. Dead (% dead) |
|---|---|---|---|---|
| 0-30 | 0 | 0 | 0 | 0 |
| 31-60 | 0 | 0 | 0 | 0 |
| 61-90 | 0 | 0 | 0 | 0 |
| 91-120 | 0 | 0 | 1(2.1) | 0 |
| 121-150 | 0 | 0 | 1(2.1) | 0 |
| 151-180 | 1(1.3) | 0 | 1(2.1) | 0 |
| 181-210 | 1(1.3) | 1(2.1) | 1(2.1) | 0 |
| 211-240 | 1(1.3) | 1(2.1) | 1(2.1) | 0 |
| 241-270 | 2(2.5) | 1(2.1) | 1(2.1) | 2(4.2) |
| 271-300 | 3(3.8) | 1(2.1) | 1(2.1) | 2(4.2) |
| 301-330 | 6(7.5) | 2(4.3) | 1(2.1) | 2(4.2) |
| 331-360 | 7(8.8) | 2(4.3) | 1(2.1) | 4(8.3) |
| 361-390 | 7(8.8) | 3(6.4) | 1(2.1) | 7(14.6) |
| 391-420 | 8(10.0) | 5(10.6) | 2(4.2) | 8(16.7) |
| 421-450 | 11(13.8) | 7(14.9) | 5(10.4) | 11(22.9) |
| 451-480 | 15(18.8) | 8(17.0) | 7(14.6) | 15(31.3) |
| 481-510 | 21(26.3) | 13(27.7) | 11(22.9) | 22(45.8)* |
| 511-540 | 29(36.3) | 14(29.8) | 16(33.3) | 29(60.4)* |
| 541-570 | 33(41.3) | 20(42.6) | 18(37.5) | 32(66.7)* |
| 571-600 | 40(50.0) | 23(48.9) | 23(47.9) | 34(70.8)* |
| 601-630 | 48(60.0) | 27(57.4) | 26(54.2) | 37(77.1)* |
| 631-660 | 54(67.5) | 33(70.2) | 33(68.8) | 40(83.3)* |
| 661-690 | 67(83.8) | 39(83.0) | 35(72.9) | 48(100)* |
| 691-720 | 70(87.5) | 41(87.2) | 40(83.3) | 48(100)* |
| 721-745 | 73(91.3) | 42(89.4) | 43(89.6) | 48(100)* |
| Total number of rats | 80(100%) | 47(100%) | 48(100%) | 48(100%) |

*Significantly different from controls by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 13-20. CUMULATIVE MORTALITY DATA OF FEMALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE
(Quast _et_ _al_., 1980a)

| Days on test | Control No. dead (% dead) | 35 ppm No. dead (% dead) | 100 ppm No. dead (% dead) | 300 ppm No. dead (% dead) |
|---|---|---|---|---|
| 0-30 | 0 | 0 | 0 | 0 |
| 31-60 | 0 | 0 | 0 | 0 |
| 61-90 | 0 | 0 | 0 | 0 |
| 91-120 | 0 | 0 | 0 | 0 |
| 121-150 | 0 | 0 | 0 | 1(2.1) |
| 151-180 | 0 | 0 | 0 | 1(2.1) |
| 181-210 | 1(1.3) | 0 | 1(2.1) | 1(2.1) |
| 211-240 | 1(1.3) | 0 | 1(2.1) | 1(2.1) |
| 241-270 | 1(1.3) | 0 | 2(4.2) | 3(6.3) |
| 271-300 | 1(1.3) | 0 | 3(6.3) | 4(8.3) |
| 301-330 | 1(1.3) | 1(2.1) | 3(6.3) | 9(18.8)* |
| 331-360 | 1(1.3) | 1(2.1) | 3(6.3) | 14(29.2)* |
| 361-390 | 1(1.3) | 3(6.3) | 5(10.4)* | 16(33.3)* |
| 391-420 | 3(3.8) | 3(6.3) | 6(12.5) | 20(41.7)* |
| 421-450 | 3(3.8) | 5(10.4) | 6(12.5) | 24(50.0)* |
| 451-480 | 6(7.5) | 7(14.6) | 11(22.9)* | 31(64.6)* |
| 481-510 | 9(11.3) | 10(20.8) | 13(27.1)* | 35(72.9)* |
| 511-540 | 11(13.8) | 12(25.0) | 17(35.4)* | 37(77.1)* |
| 541-570 | 18(22.5) | 20(41.7)* | 27(56.3)* | 42(87.5)* |
| 571-600 | 22(27.5) | 24(50.0)* | 34(70.8)* | 45(93.8)* |
| 601-630 | 34(42.5) | 27(56.3) | 38(79.2)* | 46(95.8)* |
| 631-660 | 37(46.3) | 33(68.8)* | 42(87.5)* | 46(95.8)* |
| 661-690 | 45(56.3) | 38(79.2)* | 44(91.7)* | 48(100)* |
| 691-720 | 54(67.5) | 42(87.5)* | 46(95.8)* | 48(100)* |
| 721-745 | 60(75.0) | 44(91.7)* | 47(97.9)* | 48(100)* |
| Total number of rats | 80(100%) | 48(100%) | 48(100%) | 48(100%) |

*Significantly different from controls by Fisher's Exact Probability Test, $P < 0.05$.

TABLE 13-21. HISTOPATHOLOGIC DIAGNOSES AND TUMOR INCIDENCES IN MALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE
(Quast et al., 1980a)

| Diagnoses | Tumor Incidences at Various Dose Levels*<br>0 ppm (%) | 35 ppm (%) | 100 ppm (%) | 300 ppm (%) |
|---|---|---|---|---|
| Ear Canal Gland (Zymbal gland) carcinoma | 3/80 (3.8) | 4/47 (8.5) | 3/48 (6.3) | 15/48 (31.3)<br>$P = 2.57 \times 10^{-5}$† |
| Tongue squamous cell tumors (papilloma and/or carcinoma) | 1/75 (1.3) | 2/7§ | 4/9§<br>$P = 3.10 \times 10^{-4}$† | 5/40§<br>$P = 0.019$† |
| Stomach - Nonglandular portion papilloma, squamous cell | 0/80 (0.0) | 2/46 (4.3) | 20/48 (41.7)<br>$P = 1.40 \times 10^{-10}$† | 34/47 (72.3)<br>$P = 1.59 \times 10^{-20}$† |
| Stomach - Nonglandular portion squamous cell tumors papilloma and/or carcinoma | 0/80 (0.0) | 3/46 (6.5)<br>$P = 0.047$† | 23/48 (47.9)<br>$P = 2.24 \times 10^{-12}$† | 39/47 (83.0)<br>$P = 3.95 \times 10^{-25}$† |
| Brain and/or Spinal Cord focal or multifocal glial cell proliferation suggestive of early tumor | 0/80 (0.0) | 4/47 (8.5)<br>$P = 0.017$† | 4/48 (8.3)<br>$P = 0.018$† | 8/48 (16.7)<br>$P = 2.64 \times 10^{-4}$† |
| Brain and/or Spinal Cord glial cell tumor (astrocytoma) | 1/80 (1.3) | 8/47 (17.0)<br>$P = 1.5 \times 10^{-3}$† | 19/48 (39.6)<br>$P = 7.86 \times 10^{-9}$† | 23/48 (47.9)<br>$P = 4.16 \times 10^{-11}$† |
| Brain and/or Spinal Cord glial cell tumor (benign and/or malignant) | 1/80 (1.3) | 12/47 (25.5)<br>$P = 2.27 \times 10^{-5}$† | 22/48 (45.8)<br>$P = 1.61 \times 10^{-10}$† | 30/48 (62.5)<br>$P = 1.21 \times 10^{-15}$† |

*Tumor incidence expressed as number of rats bearing certain tumor types over the number of rats examined microscopically for that particular organ.

†P-values calculated using the one-tailed Fisher Exact Test.

§All samples were not taken from all animals.

## TABLE 13-22. HISTOPATHOLOGIC DIAGNOSES AND TUMOR INCIDENCES IN FEMALE RATS MAINTAINED FOR 2 YEARS ON DRINKING WATER CONTAINING ACRYLONITRILE

(Quast *et al.*, 1980a)

| | | Tumor Incidences at Various Dose Levels* | | |
|---|---|---|---|---|
| Diagnoses | 0 ppm (%) | 35 ppm (%) | 100 ppm (%) | 300 ppm (%) |
| Mammary Gland<br>fibroadenoma/<br>adenofibroma | 56/80 (70.0) | 41/48 (85.4)<br>P = 0.037† | 39/48 (81.3) | 25/48 (52.1) |
| Mammary Gland<br>benign and/or malignant<br>mammary gland tumors | 57/80 (71.3) | 42/48 (87.5)<br>P = 0.026† | 42/48 (87.5)<br>P = 0.026† | 35/48 (72.9) |
| Ear Canal Gland (Zymbal gland)<br>adenoma | 0/80 (0.0) | 1/48 (2.0) | 3/48 (6.3)<br>P = 0.051† | 4/48 (8.3)<br>P = 0.018† |
| Ear Canal Gland (Zymbal gland)<br>carcinoma | 1/80 (1.3) | 4/48 (8.3) | 6/48 (12.5)<br>P = 0.011† | 14/48 (29.2)<br>P = $3.00 \times 10^{-6}$† |
| Ear Canal Gland (Zymbal gland)<br>benign and/or malignant tumors | 1/80 (1.3) | 5/48 (10.4)<br>P = 0.028† | 8/48 (16.7)<br>P = $1.67 \times 10^{-3}$† | 18/48 (37.5)<br>P = $2.72 \times 10^{-8}$† |
| Tongue<br>carcinoma,<br>squamous cell | 0/78 (0.0) | 1/5 | 0/3 (0.0) | 10/45 (22.2)<br>P = $2.13 \times 10^{-5}$† |
| Tongue<br>papilloma and/or carcinoma,<br>squamous cell | 0/78 (0.0) | 1/5 | 2/3 (66.7)<br>P = $9.26 \times 10^{-4}$† | 12/45 (26.7)<br>P = $2.00 \times 10^{-6}$† |
| Stomach - Nonglandular<br>papilloma, squamous cell | 1/80 (1.3) | 1/47 (2.1) | 12/48 (25.0)<br>P = $2.72 \times 10^{-5}$† | 29/48 (60.4)<br>P = $6.03 \times 10^{-15}$† |

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*Tumor incidence expressed as number of rats bearing certain tumor types over the number of rats examined microscopically for that particular organ.

†P-values calculated using the one-tailed Fisher Exact Test.

§All samples were not taken from all animals.

(continued on the following page)

TABLE 13-22. (continued)

| Diagnoses | 0 ppm (%) | Tumor Incidences at Various Dose Levels*<br>35 ppm (%) | 100 ppm (%) | 300 ppm (%) |
|---|---|---|---|---|
| Stomach - Nonglandular portion carcinoma, squamous cell | 0/80 (0.0) | 0/47 (0.0) | 0/48 (0.0) | 12/48 (25.0)<br>$P = 2.94 \times 10^{-6}$† |
| Stomach - Nonglandular portion papilloma and/or carcinoma, squamous cell | 1/80 (1.3) | 1/47 (2.1) | 12/48 (25.0)<br>$P = 2.72 \times 10^{-5}$† | 30/48 (62.5)<br>$P = 1.21 \times 10^{-15}$† |
| Small intestine cystadenocarcinoma, mucinous | 0/80 (0.0) | 1/7§ | 4/11§<br>$P = 1.23 \times 10^{-4}$† | 4/48 (8.3)<br>$P = 0.018$† |
| Brain and/or Spinal Cord focal or multifocal glial cell proliferation suggestive of early tumor | 1/80 (1.3) | 5/48 (10.4)<br>$P = 0.028$† | 4/48 (8.3) | 7/48 (14.6)<br>$P = 4.38 \times 10^{-3}$† |
| Brain and/or Spinal Cord focal or multifocal glial cell tumor (astrocytoma) | 0/80 (0.0) | 17/48 (35.4)<br>$P = 6.90 \times 10^{-9}$† | 22/48 (45.8)<br>$P = 9.15 \times 10^{-12}$† | 24/48 (50.0)<br>$P = 5.34 \times 10^{-13}$† |
| Brain and/or Spinal Cord focal or multifocal glial cell tumors (benign and/or malignant) | 1/80 (1.3) | 20/48 (41.7)<br>$P = 2.21 \times 10^{-9}$† | 25/48 (52.1)<br>$P = 2.52 \times 10^{-12}$† | 31/48 (64.6)<br>$P = 2.31 \times 10^{-16}$† |

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*Tumor incidence expressed as number of rats bearing certain tumor types over the number of rats examined microscopically for that particular organ.

†P-values calculated using the one-tailed Fisher Exact Test.

§All samples were not taken from all animals.

in all treatment groups and in females in the mid- and high-dose groups; tongue tumors in males in all treatment groups and in females in the mid- and high-dose groups; mammary gland tumors in females in the low- and mid-dose groups; tumors in the small intestine in females in the mid- and high-dose groups.

The central nervous system tumors included a statistically significant increase in the incidence of astrocytomas. There was also a statistically significant increase in the incidence of glial cell proliferations, suggestive of early tumors, which were observed most frequently in the cerebral cortex. Zymbal gland tumors were observed in the ear canal, and these tumors were usually ulcerated. In some animals, they caused displacement of the lower jaw, which resulted in the inhibition of food consumption. In the nonglandular portion of the stomach, both papillomas and carcinomas were found with a progression from hyperplasia and hyperkeratosis to papilloma and, finally, to carcinoma. Tongue tumors were diagnosed as squamous cell papillomas and carcinomas, and tumors in the small intestine were identified as cystadenocarcinomas.

### 13.5.1.1.2 Bio/Dynamics Inc. Study in Sprague-Dawley Rats (1980a)

A report entitled "A Twenty-four Month Oral Toxicity/Carcinogenicity Study of Acrylonitrile Administered to Spartan Sprague-Dawley Rats in the Drinking Water," dated June 30, 1980, was submitted to the U.S. Environmental Protection Agency by the Monsanto Company, St. Louis, Missouri. This study, conducted by Bio/Dynamics Inc. for the Monsanto Company, was designed to evaluate the toxicity/carcinogenicity of acrylonitrile. Acrylonitrile (100% pure, supplied by the Monsanto Company) was administered in the drinking water to 100 Sprague-Dawley rats of each sex at dose levels of 0, 1, and 100 ppm. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). The study was terminated early due to low survival rates; females were sacrificed at 19 months and males were sacrificed at 22 months.

Body weights for the high-dose males and females were consistently lower than the weights of controls: body weight differences between controls and treated male rats were less than 10%, while the body weight differences between controls and treated female rats were less than 8%. Body weights for the low-dose males and females were generally comparable to the controls throughout the study. Reduced water intake (test substance) was consistently noted in the high-dose group. Water intake for the low-dose group was generally comparable to the controls throughout the study. Slight but consistent decreases in hemoglobin concentration, hematocrit, and erythrocyte counts were noted for the high-dose males and females.

Histopathology evaluation revealed an increased incidence of astrocytomas of the brain and spinal cord, carcinomas and adenomas of the Zymbal gland or ear canal, and squamous cell carcinomas and papillomas of the forestomach in the high-dose males and females (Table 13-23). Increased incidences of the aforementioned tumor types were observed predominantly in animals dying or sacrificed in a moribund condition, or at sacrifice intervals after the 12th month of the study, although the increased incidences of astrocytomas of the brain and carcinomas of the Zymbal gland or ear canal were noted earlier in the high-dose female after the sixth month of the study.

In conclusion, the carcinogenic effect of acrylonitrile administered to rats in drinking water further reconfirmed the earlier findings of Dow and Litton-Bionetics study discussed later in this section.

## TABLE 13-23. TUMOR INCIDENCES IN SPRAGUE-DAWLEY RATS FED ACRYLONITRILE IN DRINKING WATER*
(Bio/Dynamics Inc., 1980a)

| Dose Level (ppm) | Sex | Brain Astrocytoma | Spinal Cord Astrocytoma | Zymbal Gland/ Ear Canal Carcinomas | Stomach Papilloma/ Carcinoma |
|---|---|---|---|---|---|
| 0 | M | 2/98 (2%) | ---§ | 1/100(1%) | 3/98 (3%) |
|  | F | 0/99 (0%) | 0/96(0%) | 0/99 (0%) | 1/100(1%) |
| 1 | M | 3/95 (3%) | ---§ | 0/91 (0%) | 3/98 (3%) |
|  | F | 1/100(1%) | 0/99(0%) | 0/95 (0%) | 4/99 (4%) |
| 100 | M | 23/97(24%)† | ---§ | 14/93(15%)† | 12/97(12%)† |
|  | F | 32/97(33%)† | 7/98(7%)† | 7/98(7%)† | 7/99(7%)† |

*Animals intentionally sacrificed at 6, 12, and 18 months after acrylonitrile administration are included in the denominator.

†Statistically significant at $P < 0.05$.

§Tissues not analyzed.

### 13.5.1.1.3 Bio/Dynamics Inc. Study in Fischer 344 Rats (1980b)

A report entitled "A Twenty-four month Oral Toxicity/Carcinogenicity Study of Acrylonitrile Administered to Fischer 344 Rats in Drinking Water," dated December 12, 1980, was submitted to the U.S. Environmental Protection Agency by the Monsanto Company, St. Louis, Missouri. This study, conducted by Bio/Dynamics Inc. for the Monsanto Company, was designed to evaluate the toxicity/ carcinogenicity of acrylonitrile. Acrylonitrile (100% pure supplied by the Monsanto Company) was administered in the drinking water to 100 Fischer 344 rats of each sex at dose levels of 1, 3, 10, 30, and 100 ppm, and the control group contained 200 animals/sex. Interim necropsies were performed at 6, 12, and 18 months (20/sex from the control group and 10/sex from each treatment group each time interval). This study was originally designed to be 24 months in duration; however, to ensure at least 10 animals/sex/group for histopathological evaluation at termination, all females were sacrificed at 23 months due to low survival. The males were continued on test until the 26th month when similar survival levels were reached. Mortality in the males and females receiving 100 ppm was markedly greater than in controls, while mortality in the 10 ppm males and females receiving 3 and 30 ppm was also somewhat greater than controls. Food consumption was comparable for all groups on a g/kg/day basis. Liquid consumption for the females receiving 100 ppm was slightly lower than controls on a ml/kg/day basis, while values for the males in this group were comparable to or greater than controls.

Slight, but generally consistent reductions in hemoglobin, hematocrit, and erythrocyte counts were noted for the females receiving 100 ppm throughout the study.

Histopathological evaluation presented in Table 13-24 revealed an increased incidence of malignant tumor-bearing animals in the groups receiving 10, 30, and

TABLE 13-24. TUMOR INCIDENCE IN FISCHER 344 RATS FED ACRYLONITRILE IN DRINKING WATER*
(Bio/Dynamics Inc., 1980b)

| Groups | Dose Level (ppm) | Sex | Brain Astrocytoma† | Spinal Cord Astrocytoma† | Ear Canal (Zymbal Gland) Papilloma/Adenoma/ Carcinoma (Squamous Cell)† | Stomach-Squamous Cell Papilloma/ Carcinoma of Forestomach† |
|---|---|---|---|---|---|---|
| IA & IB | Control | M | 2/200 (1.0) | 1/196 (0.5) | 2/189(1.0) | 0/199(0.0) |
| | | F | 1/199 (0.5) | 1/197 (0.5) | 0/193(0.0) | 1/199(0.5) |
| II | 1 | M | 2/100 (2.0) | 0/99 (0.0) | 1/97 (1.0) | 1/100(1.0) |
| | | F | 1/100 (1.0) | 0/97 (0.0) | 0/94 (0.0) | 1/100(1.0) |
| III | 3 | M | 1/100 (1.0) | 0/92 (0.0) | 0/93 (0.0) | 4/97 (4.1)§ |
| | | F | 2/101 (2.0) | 0/99 (0.0) | 2/92 (2.2) | 2/100(2.0) |
| IV | 10 | M | 2/100 (2.0) | 0/98 (0.0) | 2/88 (2.3) | 4/100(4.0)§ |
| | | F | 4/95 (4.2)§ | 1/92§ (1.0) | 4/90 (4.4)§ | 2/97 (2.1) |
| V | 30 | M | 10/99(10.1)§ | 0/99 (0.0) | 7/94 (7.4)§ | 4/100(4.0)§ |
| | | F | 6/100 (6.0)§ | 0/96 (0.0) | 5/94 (5.3)§ | 4/100(4.0)§ |
| VI | 100 | M | 21/99(21.2)§ | 4/93 (4.3)§¶ | 16/93(17.2)§# | 1/100(1.0) |
| | | F | 23/98(23.4)§ | 1/91 (1.1) | 10/86(11.6)§ | 2/97 (2.1) |

*Animals sacrificed intentionally at 6, 12, and 18 months after acrylonitrile administration are included in the denominator.

†Numbers in the parentheses are percentages.

§Statistically significant at $P < 0.05$.

¶These rats had astrocytoma in both brain and spinal cord.

#One rat had unilateral papilloma/carcinoma.

100 ppm. The observed tumors were astrocytomas of the central nervous system (brain and/or spinal cord) and squamous cell carcinomas of the ear canal, as well as mammary gland carcinomas in the females receiving 100 ppm.

In summary, the ingestion of acrylonitrile via drinking water at doses of 10, 30, and 100 resulted in an increased incidence of certain tumors. The target organ specificity (central nervous system, ear canals) confirms the similar earlier findings of the drinking water study in rats by the Dow Chemical Company.

### 13.5.1.1.4 Litton-Bionetics Study (Beliles et al., 1980)

Beliles et al. (1980) of Litton-Bionetics, Inc., sponsored by the Chemical Manufacturers Association, performed a three-generation reproductive study in Charles River rats [CRL:COBS CD (SD) BR]. These rats and their offspring ingested water containing 100 or 500 ppm acrylonitrile starting approximately 15 days post weaning and were mated after 100 days. Female rats were maintained on water containing acrylonitrile for 20 weeks; following delivery of the second litter, the animals were exposed to acrylonitrile for approximately 45 weeks. Following exposure, the animals in the three generations $F_0$, $F_1$b, and $F_2$b were sacrificed and observable masses were evaluated histologically. Results of histologic evaluations are presented in Table 13-25. The tumor incidence was low; only rats of the second generation at the high-dose level showed a significant increase in the number of tumors. The low tumor incidence was probably due to the relatively short exposure and observation period (approximately 45 weeks). This study was suggestive of tumorigenic action of acrylonitrile since the types of tumors observed were the same as the statistically highly significant tumor incidence of Quast et al. (1980a). This study provides further confirmation of the incidence of astrocytoma and Zymbal gland tumors observed by Quast et al. (1980a).

TABLE 13-25. INCIDENCE OF TUMORS OBSERVED IN RATS DURING A THREE-GENERATION REPRODUCTIVE STUDY
(Beliles *et al.*, 1980)

| | Astrocytoma Incidence Dose (ppm acrylonitrile in water) | | |
|---|---|---|---|
| Generation | 0 | 100 | 500 |
| $F_0$ | 0/19 (0%) | 1/20 (0%) | 2/25 (8%) |
| $F_1b$ | 0/20 (0%) | 1/19 (5.2%) | 4/17 (23.5%)<br>$P = 0.036$* |
| $F_2b$ | 0/20 (0%) | 1/20 (5%) | 1/20 (5%) |
| Total | 0/59 (0%) | 3/59 (5%) | 7/62 (11.2%)<br>$P = 7.8 \times 10^{-3}$* |

| | Zymbal Gland Tumor Incidence Dose (ppm acrylonitrile in water) | | |
|---|---|---|---|
| Generation | 0 | 100 | 500 |
| $F_0$ | 0/19 (0%) | 0/20 (0%) | 1/25 (4%) |
| $F_1b$ | 0/20 (0%) | 2/19 (10.5%) | 4/17 (23.5%)<br>$P = 0.036$* |
| $F_2b$ | 0/20 (0%) | 0/20 (0%) | 3/20 (15%) |
| Total | 0/59 (0%) | 2/59 (3.4%) | 8/62 (12.9%)<br>$P = 3.7 \times 10^{-3}$* |

*P-values calculated using the Fisher Exact Probability Test.

### 13.5.1.2 Gavage Studies

#### 13.5.1.2.1 Maltoni *et al*., (1977)

Maltoni *et al*. (1977) performed a cancer bioassay of acrylonitrile in which 40 Sprague-Dawley rats of each sex in both the treated and control groups were exposed to a single dose of 5 mg/kg acrylonitrile by gavage dissolved in olive oil, 3 times a week, for 52 weeks. On spontaneous death, a moderate increase in tumors of the mammary gland region and forestomach of female rats was described. Although this study was very limited, with only a single exposure level and a relatively short observation period (52 weeks), the results present further evidence for the carcinogenicity of acrylonitrile.

#### 13.5.1.2.2 Bio/Dynamics Inc. Gavage Study in Sprague-Dawley Rats (1980c)

A report entitled "A Twenty-four Month Oral Toxicity/Carcinogenicity Study of Acrylonitrile Administered by Intubation to Sprague-Dawley (Spartan) Rats" was submitted to the U.S. Environmental Protection Agency, June 30, 1980, by the Monsanto Company, St. Louis, Missouri. This study was conducted by Bio/Dynamics Inc. for the Monsanto Company. In this study, acrylonitrile (100% pure*) was administered by intubation to Sprague-Dawley (Spartan) rats (100/sex/group) at three dose levels of 0, 0.10, and 10.0 mg/kg/day, 5 days/week. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). This study was originally designed to terminate at 24 months; however, because only 10 and 13 high-dose males and females, respectively, were alive by 20 months, all surviving animals in all groups were terminated during the 20th month to ensure at least 10 animals/sex for histopathological evaluation. The body weights of high-dose group males were consistently slightly lower than control.

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*Supplied by Monsanto Company, Texas City, Texas.

Histopathological evaluations presented in Table 13-26 show that there were statistically significant increased incidences in tumors of the brain and ear canal (Zymbal gland) in both high-dose males and females. Stomach and intestinal tumors were observed only in high-dose males, and mammary gland tumors were observed in high-dose females. Statistically significant tumor incidences were not observed in low-dose groups either in males or females.

In summary, acrylonitrile administered orally via intubation produces a carcinogenic effect in Sprague-Dawley rats at the following tumor sites: brain, Zymbal gland, mammary gland, stomach, and intestine.

### 13.5.1.3 Inhalation Studies

#### 13.5.1.3.1 Maltoni *et al.*, (1977)

Maltoni *et al.* (1977) exposed Sprague-Dawley rats to atmospheres containing 5, 10, 20, and 40 ppm acrylonitrile 4 hours/day, 5 days/week, for 12 months. The rats were maintained for their entire lifetime. Histological examination of the selected tissues were made. The incidence of tumors observed in this study is shown in Table 13-27. Slight increases were observed in mammary gland tumors of males and females, the forestomach of males, and the skin of females. Maltoni *et al.* (1977) claimed that these results indicated a "border-line carcinogenic effect." The detection power (sensitivity) of this assay was low because of the low concentration of acrylonitrile used and the relatively short exposure period (12 months).

#### 13.5.1.3.2 Dow Chemical Company (Quast *et al.*, 1980b)

A second inhalation study of acrylonitrile was conducted by Dow Chemical Company, sponsored by the Chemical Manufacturers Association. In this study, 100 male and female Sprague-Dawley rats (Spartan substrain) were exposed to 0,

### TABLE 13-26. TUMOR INCIDENCE IN RATS FED ACRYLONITRILE ORALLY BY INTUBATION (Bio/Dynamics Inc., 1980c)

| Dose Level mg/kg/day | Sex | Brain Astro-cytoma | Spinal Cord Astrocytoma | Ear Canal (Zymbal Gland) Squamous Cell Carcinomas | Stomach Carcinoma/ Papilloma | Intestine | Mammary Gland |
|---|---|---|---|---|---|---|---|
| 0 | M | 2/100 (2%) | 0/94 (0%) | 1/96 (1%) | 2/99 (2%) | 0/100 (0%) | ---† |
| | F | 1/99 (1%) | 0/100(0%) | 0/85 (0%) | 2/99 (2%) | ---† | 7/101 (7%) |
| 0.10 | M | 0/97 (0%) | 0/93 (0%) | 0/93 (0%) | 6/97 (6%) | 1/100 (1%) | ---† |
| | F | 2/100 (2%) | 0/95 (0%) | 0/94 (0%) | 4/99 (4%) | ---† | 6/100 (6%) |
| 10.0 | M | 16/98 (16%)* | 1/97 (1%) | 10/96(10%)* | 40/99(40%)* | 6/100 (6%)* | ---† |
| | F | 17/100(17%)* | 1/99 (1%) | 9/94 (10%)* | 17/99(17%) | ---† | 22/101(22%)* |

*Statistically significant at $P < 0.05$.

†Tissues not analyzed.

TABLE 13-27. TUMOR INCIDENCE IN RATS FOLLOWING INHALATION OF ACRYLONITRILE
(Maltoni *et al.*, 1977)

| Tumor Type (sex) | Number of Animals with Tumors (%) | | | | |
|---|---|---|---|---|---|
| | Exposure Concentration of Acrylonitrile (ppm) | | | | |
| | 0 | 5 | 10 | 20 | 40 |
| Number of animals of each sex | 30 | 30 | 30 | 30 | 30 |
| Mammary tumors (female) | 5(16) | 10(33) | 7(23) | 10(33) | 7(23) |
| Mammary tumors (male) | 1(3) | 0(0) | 1(3) | 4(13)<br>P = 0.17* | 4(13)<br>P = 0.17* |
| Zymbal gland (female) | 0(0) | 0(0) | 1(3) | 1(3) | 0(0) |
| Zymbal gland (male) | 0(0) | 0(0) | 1(3) | 0(0) | 0(0) |
| Encephalic tumors (female) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| Encephalic tumors (male) | 0(0) | 0(0) | 0(0) | 1(3) | 2(7) |
| Forestomach papillomas (female) | 0(0) | 1(3) | 2(7) | 1(3) | 0(0) |
| Forestomach papillomas (male) | 0(0) | 1(3) | 2(7) | 0(0) | 3(10) |
| Skin carcinomas (female) | 0(0) | 4(13)<br>P = 0.056* | 1(3) | 1(3) | 1(3) |
| Skin carcinomas (male) | 0(0) | 0(0) | 0(0) | 0(0) | 1(3) |
| Uterine carcinomas (female) | 1(3) | 3(10) | 1(3) | 2(7) | 1(3) |
| Total tumors (female) | 9(30) | 17(57) | 11(36) | 14(47) | 8(27) |
| Total tumors (male) | 4(13) | 1(3) | 10(33) | 13(43) | 12(40) |

*P-values calculated using the one-tailed Fisher Exact Test.

20, or 80 ppm of acrylonitrile 6 hours per day, 5 days per week, for 2 years, except during weekends or holidays. The acrylonitrile used for this study was produced by E.I. du Pont de Nemours and Company, Inc. Its stability and impurities are summarized in Table 13-28. The nominal concentration of acrylonitrile in the exposure chamber was calculated from the rate at which the liquid test material was dispensed and the rate of airflow through the chamber. The target concentrations and chamber concentrations are shown in Table 13-29.

During the course of the 2-year study, hematology, urinalysis, and clinical chemistry determinations were performed at periodic intervals. The results of these determinations indicated that acrylonitrile did not have an adverse effect on bone marrow, kidney, or liver functions in either male or female rats. During the first 6 months of the study, the exposed rats drank more water and appeared to excrete a lower specific gravity urine than control rats.

In lifetime observations of male and female rats exposed to acrylonitrile vapors, toxic effects, characterized by decrease in body weight and early mortality, were observed. The cumulative mortality data are shown in Tables 13-30 and 13-31.

Microscopic examination of tissues revealed a treatment-related statistically significant incidence of tumors in the central nervous system, ear canal gland (Zymbal gland), tongue, small intestine, and mammary gland. In male and female rats, statistically significant increased incidences were observed only at the 80 ppm dose levels with the exception of glial cell tumors of the central nervous system which were also increased in female rats at 20 ppm. Detailed results are presented in Table 13-32. However, these results are based on data presented in the Quast et al. (1980b) inhalation study and do not reflect separation of individual animals having one or more tumor types. The CAG has requested these individual data from Dr. Quast and the Chemical Manufacturers Association.

TABLE 13-28. STABILITY AND TRACE IMPURITY ANALYSIS OF THE ACRYLONITRILE LIQUID TEST MATERIAL (Quast et al., 1980b)

| | Sample #1 Dated 1/27/76 | Sample #2 Dated 5/24/76 | Sample #3 Dated 11/19/76 | Sample #4 Dated 3/21/77 | Sample #5 Dated 9/7/77 | Sample #6 Dated 1/13/78 |
|---|---|---|---|---|---|---|
| Refractive Index (D), 25.00°C | 1.3908 | 1.3908 | 1.3908 | 1.3901 | 1.3904 | 1.3909 |
| P-Methoxy phenol (MEHQ) Inhibitor, ±1 ppm | 34 | 32 | 35.5 | 34.5 | 38.8 | 41.7 |
| Methacrylonitrile by GLC, ±2 ppm | 74.5 | 75 | 74.8 | 47 | 33 | 56.5±1.5 |
| Other Compounds by GLC | None | None | None | None | None | ---* |
| Acetone by GLC, ppm | None | None | None | 88±5 | 195±5 | 131±7 |
| Volatiles by Mass Spec. | Water Only† | Water Only† | Water Only† | Water Only† | Water Only† | AN Only |
| Peroxides as ppm $H_2O_2$ (Polargraphic Data) | 5 | 1 | 4.5 | 3.8 | 0.2§ | N.D. |
| Metals by Atomic Emission with their Lower Detection Limit in Parentheses, ppm¶ | | | | | | |
| Metal | | | | | | |
| Fe | 0.5[a] | 0.8[a] | 0.8(0.2) | 1[a] | N.D.(1) | 1(1) |
| Ni | N.D.(0.2) | N.D.(0.2) | N.D.(0.2) | N.D.(1) | N.D.(1) | N.D.(1) |
| Cu | 0.1[a] | 0.1[a] | 0.1(0.1) | N.D.(0.2) | N.D.(0.2) | N.D.(0.2) |
| Al | N.D.(0.2) | N.D.(0.2) | 0.4(0.2) | b | N.D.(1) | N.D.(1) |
| Ca | 0.7 | 1.0[a] | 0.8(0.2) | b | N.D.(1) | 1(1) |
| Si | b | b | c(0.2) | b | b | b |
| Mg | N.D.(0.2) | N.D.(0.2) | N.D.(0.2) | N.D.(0.1) | N.D.(0.1) | N.D.(0.1) |
| Mn | N.D.(0.1) | N.D (0.1) | 0.1(0.1) | N.D.(0.5) | N.D.(0.5) | N.D.(0.5) |
| Pb | N.D.(0.5) | N.D.(0.5) | N.D.(0.5) | N.D.(3) | N.D.(3) | N.D.(3) |
| Cr | N.D.(0.2) | N.D.(0.2) | N.D.(0.1) | N.D.(0.5) | N.D.(1) | N.D.(1) |
| Sn | N.D.(0.2) | N.D.(0.2) | N.D.(0.2) | b | N.D.(1) | N.D.(1) |
| Zn | N.D.(0.5) | N.D.(0.5) | c(0.5) | b | N.D.(3) | N.D.(3) |
| Ti | N.D.(0.2) | N.D.(0.2) | N.D.(0.2) | N.D.(1) | N.D.(1) | N.D.(1) |

Data is given in ppm on a volume/volume basis for the liquid test material.

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*Small unidentified peak ahead of acetone estimated to be about 25 ppm.

†Sensitivity is limited to about 100 ppm.

§Detection limit is 0.2.

¶The analysis of the sample analyzed is accurate to ± 10-15% of amount present.

[a] Lower detection limit was not specified.

[b] No analysis was made for this metal.

[c] Metal was detected, but unmeasurable.

N.D. - Not Detectable.

TABLE 13-29. CHAMBER CONCENTRATIONS OF ACRYLONITRILE VAPORS (Quast et al., 1980b)

| Target Concentration (ppm) | Exposure Group<br>20 ppm | <br>80 ppm |
|---|---|---|
| Analytical Concentration* (ppm) X ± S.D. | 20.0±1.9 | 80.0±5.9 |
| Range of Daily Analytical Concentration (ppm) | 7.3-35.0 | 45.2-106.0 |
| Nominal Concentration† (ppm) X ± S.D. | 20.7±2.6 | 90.1±7.3 |
| Range of Daily Nominal Concentration | 11.00-37.8 | 59.5-138.2 |
| Average Analytical Concentration/ Average Nominal Concentration | 0.97 | 0.89 |
| Number of Exposure Days Within ±10% of Target Concentration | 433 | 470 |
| Number of Exposure Days Within ±25% of Target Concentration | 64 | 33 |
| Total Exposure Days | 507§ | 508 |
| Total Number of Exposure Analyses | 1484 | 1494 |

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*Data represents mean (X) ± standard deviation (S.D) of daily 6-hour time-weighted average for the total number of exposure days.

†Data represents mean (X) ± standard deviation (S.D.) of daily 6-hour nominal concentration for the total number of exposure days.

§The 20 ppm exposure group had one less exposure day than the 80 ppm exposure group as a result of mechanical failure in the ventilation system of the 20 ppm exposure chamber.

### TABLE 13-30. CUMULATIVE MORTALITY DATA OF MALE RATS EXPOSED BY INHALATION FOR 2 YEARS TO ACRYLONITRILE VAPORS*
(Quast *et al.*, 1980b)

| Days on Test | Exposure Concentration<br>0 ppm | <br>20 ppm | <br>80 ppm |
|---|---|---|---|
| 0-30 | 0 | 1 | 0 |
| 31-60 | 0 | 1 | 0 |
| 61-90 | 0 | 1 | 1 |
| 91-120 | 0 | 2 | 1 |
| 121-150 | 1 | 2 | 1 |
| 151-180 | 2 | 3 | 2 |
| 181=210 | 2 | 3 | 6 |
| 211-240 | 2 | 4 | 12† |
| 241-270 | 2 | 5 | 13† |
| 271-300 | 2 | 5 | 14† |
| 301-330 | 3 | 6 | 16† |
| 331-360 | 3 | 6 | 18† |
| 361-390 | 4 | 8 | 19† |
| 391-420 | 6 | 9 | 22† |
| 421-450 | 11 | 12 | 24† |
| 451-480 | 14 | 15 | 28† |
| 481-510 | 19 | 26 | 39† |
| 511-540 | 23 | 34 | 47† |
| 541-570 | 27 | 38 | 56° |
| 571-600 | 35 | 47 | 63† |
| 601-630 | 43 | 59† | 76† |
| 631-660 | 62 | 68 | 83† |
| 661-690 | 71 | 72 | 85† |
| 691-720 | 78 | 81 | 94† |
| 721-735 | 82 | 86 | 96† |
| Total Number of Rats | 100 | 100 | 100 |

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*Data listed as number dead which is equal to percent dead.

†Significantly different from control by Fisher's Exact Probability Test, $P < 0.05$.

## TABLE 13-31. CUMULATIVE MORTALITY DATA OF FEMALE RATS EXPOSED BY INHALATION FOR 2 YEARS TO ACRYLONITRILE VAPORS*

(Quast *et al.*, 1980b)

| Days on Test | Exposure Concentration<br>0 ppm | <br>20 ppm | <br>80 ppm |
|---|---|---|---|
| 0-30 | 1 | 0 | 0 |
| 31-60 | 1 | 0 | 0 |
| 61-90 | 1 | 0 | 0 |
| 91-120 | 1 | 0 | 0 |
| 121-150 | 1 | 0 | 0 |
| 151-180 | 1 | 0 | 0 |
| 181-210 | 1 | 1 | 1 |
| 211-240 | 1 | 1 | 2 |
| 241-270 | 3 | 1 | 4 |
| 271-300 | 5 | 1 | 6 |
| 301-330 | 5 | 2 | 9 |
| 331-360 | 7 | 2 | 11 |
| 361-390 | 9 | 3 | 19† |
| 391-420 | 11 | 5 | 27† |
| 421-450 | 14 | 10 | 33† |
| 451-480 | 14 | 14 | 41† |
| 481-510 | 19 | 22 | 57† |
| 511-540 | 26 | 31 | 71† |
| 541-570 | 34 | 36 | 80† |
| 571-600 | 36 | 43 | 88† |
| 601-630 | 50 | 54 | 94† |
| 631-660 | 63 | 70 | 98† |
| 661-690 | 66 | 81† | 98† |
| 691-720 | 71 | 88† | 99† |
| 721-735 | 78 | 91† | 99† |
| Total Number of Rats | 100 | 100 | 100 |

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*Data listed as number dead which is equal to percent dead.

†Significantly different from control by Fisher's Exact Probability Test, $P < 0.05$.

## TABLE 13-32. TUMOR INCIDENCE IN SPRAGUE-DAWLEY RATS EXPOSED TO ACRYLONITRILE BY INHALATION (Quast *et al*., 1980b)

| Diagnoses | 0 ppm | 20 ppm | 80 ppm |
|---|---|---|---|
| Males | | | |
| Zymbal gland tumors of external ear canal (benign and malignant) | 2/100 | 4/100 | 11/100<br>(P = 0.009)* |
| Small intestine tumors (benign and malignant) | 2/99 | 2/20 | 15/98<br>(P = $7.03 \times 10^{-4}$)* |
| Brain and/or spinal cord glial cell tumors (benign and malignant) | 0/100 | 4/99 | 22/99<br>(P = $5.71 \times 10^{-8}$)* |
| Tongue-squamous cell tumors (benign and malignant) | 1/96 | 0/14 | 7/89<br>(P = 0.0251)* |
| Females | | | |
| Zymbal gland tumors of external ear canal (benign and malignant) | 0/100 | 1/100 | 11/100<br>(P = $3.65 \times 10^{-4}$)* |
| Mammary gland fibroadenoma/adenofibroma | 79/100 | 96/100<br>(P = $2.06 \times 10^{-4}$)* | 75/100 |
| Mammary gland adenocarcinoma | 9/100 | 8/100 | 20/100<br>(P = 0.022)* |
| Mammary gland tumors (benign and malignant) | 88/100 | 96/100 | 85/100 |
| Brain and/or spinal cord glial cell tumors (benign and malignant) | 0/100 | 8/100<br>(P = 0.003) | 21/100<br>(P = $3.32 \times 10^{-7}$)* |

*P-values calculated by Fisher's Exact Probability Test.

In summary, in the Dow study acrylonitrile induced a statistically significant increased incidence of tumors in male and female rats following exposure by inhalation.

### 13.5.1.4 Cell Transformation Study

#### 13.5.1.4.1 Parent and Casto (1979)

Parent and Casto (1979) reported the effect of acrylonitrile on Syrian golden hamster embryo cells (HEC), and found that acrylonitrile (ACN) transforms cells in culture and enhances the transformation of cells previously affected with simian adenovirus SA7, a colony transforming oncogenic virus.

ACN from Aldrich Chemical Co. (Milwaukee, Wis.) was used in this experiment. The purity was stated to be greater than or equal to 99%. The impurities included about 0.3% water, less than 0.5% acetonitrile, and 30 to 45 ppm 1-hydroxy-4-methoxybenzene. In this experiment cultures of primary Syrian golden HEC were prepared by trypsinization of decapitated and eviscerated embryos after 13-14 days of gestation. Cells were plated into 60-mm-diameter Lux plastic dishes at a density of $5 \times 10^6$ cells/dish with modified Dulbecco's medium and 10% fetal bovine serum (Reheis Chemical Co., Kankakee, Ill.) and incubated at 37°C for 3 days in 5% $CO_2$. ACN was dissolved in 100 mg acetone/ml and diluted in complete medium to give the final concentrations.

In this viral transformation enhancement assay, HEC were exposed to ACN in concentrations of 0, 25, 50, 100, and 200 ug/ml. Treatment of HEC with ACN for 18 hours before SA7 inoculation resulted in only slight but significant enhancement to 1.8-fold (Table 13-33). When cells were treated with ACN 5 hours after they were inoculated with virus (Table 13-33), a significant enhancement of 8.9- and 8.4-fold was observed at 200 and 100 ug ACN/ml, respectively. Treatment with 200 ug ACN/ml reduced the cloning efficiency to less than 10%, but the number of SA7 foci only decreased from 41 in control to 26 in treated cells. The enhancement found when cells were chemically treated after virus inoculation was observed with several other chemicals.

TABLE 13-33. ENHANCEMENT OF SA7 TRANSFORMATION BY TREATMENT OF HEC WITH ACN*
(Parent and Casto, 1979)

| Time of ACN treatment | ACN ug/ml | Surviving fraction† | SA7 foci§ | Enhancement ratio¶ |
|---|---|---|---|---|
| 18 hr before SA7 | 200 | 0.08 | 1 | 0.3 |
| | 100 | 0.34 | 32 | 1.8 |
| | 50 | 0.62 | 41 | 1.3 |
| | 25 | 0.75 | 45 | 1.2 |
| | 0 | 1.00 | 50 | 1.0 |
| 18 hr before SA7 | 200 | 0.18 | 6 | 1.6 |
| | 100 | 0.46 | 19 | 2.1 |
| | 50 | 1.07 | 31 | 1.5 |
| | 25 | 1.02 | 37 | 1.8 |
| | 0 | 1.00 | 20 | 1.0 |
| 5 hr after SA7 | 200 | 0.07 | 26 | 8.9 |
| | 100 | 0.21 | 75 | 8.4 |
| | 50 | 0.60 | 37 | 1.5 |
| | 25 | 0.69 | 41 | 1.4 |
| | 0 | 1.00 | 41 | 1.0 |

---

*Chemical dilutions were added to mass cultures of HEC 18 hr before or 5 hr after treatment with SA7. Virus was absorbed 3 hr, and the cells were transferred for survival (500-700 cells/dish) and for transformation assays (200,000-300,000 cells/dish).

†Determined from plates receiving 500-700 cells. Number of colonies from virus-treated and chemically-treated cells was divided by the number of colonies from virus-inoculated control cells to give the surviving fraction. Cloning efficiency of control cells was 10-15%.

§Number of foci from $10^6$ plated cells.

¶Enhancement ratio was determined by dividing the TF of untreated cells (TF = SA7 foci x reciprocal of the surviving fraction) by that obtained from control cells. Underlined values are statistically significant at the 5% level.

When HEC were treated for 6 days with ACN (chemical transformation) without added virus, foci of morphologically transformed cells were observed that were similar to those described previously with known chemical carcinogens. At 100 ug ACN/ml, three foci were observed on nine dishes and two foci on six dishes at 50 ug ACN/ml; BP treatment resulted in three foci on four dishes at 1.25 ug/ml and two foci on ten dishes at 0.62 ug/ml (Table 13-34). No foci were observed on medium or solvent control dishes.

The observation that acrylonitrile transforms cells adds support to the animal and human evidence that acrylonitrile may be carcinogenic.

TABLE 13-34. TRANSFORMATION OF HEC BY ACN
(Parent and Casto, 1979)

| Treatment* | ACN ug/ml | Surviving fraction† | Foci/dishes |
|---|---|---|---|
| ACN | 100 | 0.06 | 3/9 |
| | 50 | 0.76 | 2/6 |
| | 25 | 0.84 | 0/6 |
| | 12 | 1.06 | 0/5 |
| BP | 1.25 | 0.78 | 3/4 |
| | 0.62 | 0.94 | 2/10 |
| Control | --- | 1.00 | 0/7 |

*Chemicals were added to tertiary HEC plated 24 hr earlier with 50,000 (transformation) or 1,000 (survival) cells/dish. Fresh medium with chemical was added after 3 days and removed after 6 days. Colonies were fixed and stained at 9 days for survival assays; focus assays for transformation were done 25 days after treatment was indicated.

†Determined from dishes receiving 1,000 cells. Number of colonies from treated plates was divided by the number from control dishes.

### 13.5.2 Epidemiologic Studies

#### 13.5.2.1 O'Berg (1980)

There are no community epidemiologic studies available that attempt to demonstrate an association between exposure to ambient levels of acrylonitrile and the development of disease. However, an occupational epidemiologic study that involved workers exposed to acrylonitrile at a Du Pont textile fibers plant in Camden, South Carolina was conducted by Maureen T. O'Berg of E.I. du Pont de Nemours and Co., Inc. (O'Berg, 1980). The study cohort was 1,345 male employees "identified as having had potential exposure to acrylonitrile at some time between start-up in 1950 and 1966." The 1966 cutoff date allowed for a minimum 10-year follow-up through the end of 1976. Expected numbers were stated to be based both on company and national rates. The analyses presented in the paper, however, derive expected number of cases based only on company rates, ignoring the possible effects of other chemicals on this "control" cohort.* The analyses consider calendar time, payroll classification, occupation, duration of exposure, latency, and severity of exposure. The severity of exposure levels were designated as high, medium, and low. Du Pont representatives agreed that 20 ppm, 10 ppm, and 5 ppm might be used to represent the designated classification of high, medium, and low exposure levels. This was documented in a trip report by Jane Brown, Industrial Hygienist, National Institute for Occupational Safety and Health (NIOSH, 1978).

Overall, 25 cases of cancer occurred, with 20.5 expected based on company rates. Of these 25 cases, eight were respiratory cancer cases versus 4.4 expected. Excesses were found primarily among wage roll employees who had worked during plant start up, 1950 to 1952, and had been exposed for at least 6 months. For these employees there were 8 cases of respiratory cancer versus 2.6 expected ($P < 0.01$). Furthermore, most of this above excess occurred during the latest follow-up period, 1970 to 1976, when there were six cases of respiratory cancer versus 1.5 expected ($P < 0.01$). Total cancer cases in this latest follow-up period for this group were also significant, 17 versus 5.6 ($P < 0.01$).

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*While this paper also attempts to compare observed and expected deaths, there is an inconsistent result. According to O'Berg, 4.4 respiratory cancer cases were expected in this cohort using the Du Pont controls. However, using the same controls, O'Berg predicts 6.1 respiratory cancer deaths. A telephone conversation with O'Berg confirmed that this inconsistency was due to different methods of following the cohort for cases and deaths. Cases were described only for active employees, while deaths included retirees.

A trend toward increased risks was seen not only with increased follow-up time but also with severity of exposure. In wage roll workers with at least moderate exposure and probable latent period of at least 15 years, the observed and expected numbers of cancer cases was 13 and 5.5, respectively. Furthermore, half of this excess cancer was respiratory cancer, 5 versus 1.4 ($P < 0.05$). Thus, this study provides some evidence that acrylonitrile is carcinogenic to humans.

However, because of the known relationship between smoking and lung cancer, further analysis is attempted concerning the role of smoking behavior in these findings. (Seven of the eight lung cancer cases were reported to be smokers by their supervisors or associates; the eighth was unknown.) Dr. Bruce Karrh of Du Pont stated that there were pathology slides for five of the eight respiratory cancer cases. He further stated that these were identified as four squamous cell carcinomas and one oat cell carcinoma. Of the three remaining respiratory cancer cases for whom slides were unavailable, he stated that two were bronchogenic and the other unknown. These cell types are generally believed to be associated with both chemicals and smoking by most pathologists.

In an attempt to investigate the impact of smoking on the risk of developing lung cancer in this population, the Du Pont Company provided additional data (letter from Sidney Pell, Du Pont, July 23, 1980) to the Carcinogen Assessment Group (CAG) regarding the smoking habits of 32 of the 36 cancer cases reported on in this plant (some of these were not in the study cohort), as well as data on the smoking habits of a matched group of non-cases in the plant. Of the 32 cancer cases, 22 were cancers other than lung, and 16 or 73% of the non-lung cancer cases were smokers. The smoking habits of the matched group of 36 noncancer controls from the same plant were also provided. They were matched on a three to one basis to certain selected cancers* occurring in the study group on the basis of age, payroll classification, date of first exposure, and date of termination. It was found that 25 or 69% of the 36 cases were smokers in this group.

Based on this information, we can estimate that 70% of the plant population were smokers, and 30% nonsmokers. Of the 70% who were smokers, we will assume that 50% were "moderate" smokers while 20% were "heavy" smokers based on figures by Axelson (1978). We will assume also that the relative risk of lung cancer is 1, 10, and 20, respectively, for the nonsmokers, moderate smokers, and heavy smokers (Doll and Hill, 1952; Hammond, 1975). Using the method of Axelson (1978) to adjust for smoking differences in cohorts, the relative incidence of lung cancer in the plant population is

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*The author selected only cancer cases that have a high correlation with cigarette smoking for his case control study. The purpose was to test whether cigarette smoking was different in the matched non-cases selected from the same plant compared to the cases. These cases were eight lung cancers, one esophageal, one nasopharyngeal, and two bladder cancers. The resulting test statistic ($X^2$) was nonsignificant, indicating that there was no reason to suspect that there was an uneven distribution of cigarette smoking in the cases compared to the matched non-cases.

$$(1) \qquad I_p = 1(0.30)I_o + 10(0.50)I_o + 20(0.20)I_o = 9.3\ I_o$$

where $I_o$ equals the risk of lung cancer in a nonsmoking population.

Based on a nationwide survey (U.S. Health, Education, and Welfare, 1973) which found that 62% of the male blue collar workers smoked in 1966, we assume that the overall company population had 40% nonsmokers. In addition, we assume that the distribution of moderate and heavy smokers is the same as above, so that moderate and heavy smokers constitute 43% and 17% of the population respectively. The computed incidence of lung cancer in this comparison population is then

$$(2) \qquad I_g = 1(0.40)I_o + 10(0.43)I_o + 20(0.17)I_o = 8.1\ I_o$$

where $I_g$ is the incidence in the company population. Hence, the relative contribution of smoking to the risk of lung cancer in the study cohort is the ratio of the two incidence rates:

$$(3) \qquad I_p/I_g = 9.3\ I_o/8.1\ I_o = 1.15$$

Therefore, because of the slightly higher proportion of smokers in the study cohort relative to the reference company population, the number of respiratory cancer cases would be about 15% higher than the 1.4 cases expected without considering smoking differences, or 1.4 x 1.15 = 1.61 cases. Assuming a Poisson distribution of cases, the probability of seeing 5 cases or more when only 1.61 are expected is only 0.024. Therefore, after the adjustment for smoking differences, the respiratory cancer rate in the study cohort exposed to acrylonitrile for over 6 months is significantly higher than that of the reference population.

In an attempt to assess whether the excess respiratory cancer rates could be ascribed to a higher ratio of heavy to moderate smokers in the acrylonitrile-exposed cohort than that in the reference population, the following calculation was done. In equation (1) for $I_p$ the ratio of heavy to moderate smokers was increased from $0.2/0.5 = 1/2.5$, keeping the total fraction of smokers at 0.7, until the point was reached where the probability of observing five cases or more giving the expected number, $1.4 \times (I_p/I_g)$, was increased from 0.024, as above, to 0.05. The result is that in order for a higher ratio of heavy to moderate smokers in the cohort to account (at the 0.05 level of significance) for the observed excess respiratory cancer rate, that ratio would have to be 1.6/1. Our judgment is that such a marked excess of heavy versus moderate smokers would not likely occur in the acrylonitrile workers, since for blue collar workers generally there are about 2.5 times more moderate smokers than heavy smokers.

In conclusion, the observations by O'Berg of a statistically significant excess of respiratory cancer in workers exposed to acrylonitrile and followed up for more than 10 years constitute significant evidence that acrylonitrile is likely to be a human carcinogen, although smoking, at least as a contributing factor, cannot be completely ruled out at this time.

#### 13.5.2.2 Delzell and Monson (1982)

Delzell and Monson (1982) conducted a cohort mortality study of 327 white male employees of a rubber manufacturing plant in Akron, Ohio who were employed for at least 2 or more years between January 1, 1940 and July 7, 1971 in two areas with potential exposure to acrylonitrile. The first area was a nitrile rubber manufacturing operation. Butadiene and styrene were present in this area between 1938 and 1949 while vinyl pyridine has been present since 1949. The latex that was produced in closed vessels was "blown down" into tanks after excess monomers were removed. In the second area, latex was coagulated and dried into solid rubber. From 1938 to 1960, the nitrile rubber was washed on an open mill and dried in vacuum ovens. After 1960, this job was done by extruder drying. Delzell and Monson did not report the levels of acrylonitrile that these workers were exposed to while on the job.

Follow-up was continued until July 1, 1978, according to the authors, but the extent of success is not indicated. The authors state that the follow-up was "identical" to that of a recently published study by the same authors. The authors do indicate that anyone without a death record prior to July 1, 1978 was assumed alive on that date. Expected deaths were generated based on U.S. age and calendar time specific white male mortality. Overall, 74 deaths occurred in this group compared to 89.5 expected, a deficit readily attributable to the healthy worker effect. On the other hand, the risk of death due to cancer was somewhat elevated, with 22 observed versus 17.9 expected. Most of this excess was due to lung cancer deaths which were nonsignificantly higher, with 9 observed versus 5.9 expected. Only in workers employed for 5 to 14 years and followed for at least 15 years, did a significantly elevated risk of lung cancer appear (4 observed versus 0.8 expected $P < 0.01$). However, if workers who were employed for more than 14 years and followed for at least 15 years are added to the latter group, not an unreasonable consideration since presumably they are most exposed, the risk of lung cancer was actually reduced but still significant (4 observed versus 1.4 expected, $P < 0.05$).

The excessive risk of lung cancer in this group may not be entirely due to exposure to acrylonitrile, but may be partially reflective of exposures to other substances within the same environment, i.e., the author reports that one lung cancer victim had worked for 17 years in the curing division of the company prior to employment in the acrylonitrile area, but the authors did not elaborate further. Additionally, no discussion of the effects of smoking on lung cancer is included.

However, the possibility that the excess risk of lung cancer is due to acrylonitrile cannot be dismissed.

### 13.5.2.3 Thiess _et_ _al_. (1980)

A cohort mortality study of 1,469 workers from 12 factories located in the Federal Republic of Germany was conducted by Thiess _et_ _al_. (1980). These 12 factories are part of the BASF company. The BASF company produces no acrylonitrile but buys acrylonitrile in order to produce styrene-acrylonitrile and acrylonitrile butadiene-styrene polymers as well as organic intermediate products. The processing methods differ from factory to factory. No historic exposure data for acrylonitrile exists according to the authors.

The population at risk was defined as all workers who were employed for over 6 months in acrylonitrile processing presumably from time of first use of acrylonitrile (around 1956) until the cut-off date of May 15, 1978. Included were 1,081 German workers and 338 "foreigners" (nationality not given). Follow-up was 98% complete on the German workers but only 56% complete on the foreign segment. This left about 170 lost to follow-up or about 12% of the work force.

Expected deaths were generated based upon mortality in three areas of Germany, the city of Ludwigshafen, the state of Rheinhessen-Pfalz, and the

Federal Republic of Germany (FRG). A total of 89 deaths was observed compared to 92.3, 96.4, and 99.0 expected deaths, respectively, in the three geographic entities cited. Twenty-seven deaths from malignant tumors were observed compared to 20.5 expected based upon rates of the FRG. The most striking finding was a statistically significant higher risk of cancer of the lung (11 observed versus 5.65 expected based on FRG rates, $P < 0.05$; or 5.92 expected based on rates of Rheinhessen-Pfalz, $P < 0.05$). The authors recalculated their results for the FRG only, with the 78 members of one factory excluded from the group of 12 factories whom they said had "contact with substances since proven to be carcinogenic." Not only did an excessive significant risk remain with respect to lung cancer (9 observed versus 4.37 expected based on FRG rates, $P < 0.05$), but also a significant excess risk of cancer of the lymphatic system was seen (4 observed versus 1.38 expected, $P < 0.05$).

These results are questionable. First, the members of this cohort were apparently exposed to a number of different carcinogens, i.e., vinyl chloride, distillation residues containing polycyclic hydrocarbons, cadmium, B-napthylamine, dimethylsulfate, and epichlorohydrin. Several of these have been associated with a higher risk of lung cancer. Second, all lung cancer victims were found to be smokers, while not all lung cancer victims were proven to be exposed to acrylonitrile, according to the author.

On the other hand, the lung cancer risk estimated from this study may actually be an underestimate of the true risk for the following reasons:

1. Lumping together workers from 12 different factories who may be subjected to differing levels of exposures to acrylonitrile could have led to an underestimate of risk by the inclusion of minimally exposed or unexposed members.

2. The healthy worker effect would contribute to an underestimate of risk.

3. Insufficient follow-up on a relatively youthful cohort did not allow enough latency in the segments of the cohort most likely to exhibit a true estimate of risk. Only 447.1 person-years were accumulated in members over 64 years of age.

4. Underascertainment of vital status may have resulted in an undercount of observed deaths. Twelve percent were lost to follow-up. Person-years have been accumulated for each of the lost individuals until the last date known to be alive.

Although, there are many limitations to this study, it is possible that exposure to acrylonitrile may indeed be related to an excessive risk of lung cancer and cancer of the lymph system as well as to other carcinogens present in the workplace.

### 13.5.2.4 Werner and Carter (1981)

Werner and Carter (1981) conducted a cohort mortality study of 934 men who worked on the polymerization of acrylonitrile and spinning of acrylic fiber at six different factories sometime between 1950 and the end of 1968 for a minimum of one year. Two of the six factories were located in Scotland and Northern Ireland; the remainder were located in England and Wales. The cohort was followed through the end of 1978, which resulted in a vital status ascertainment near 100%. Expected deaths were calculated based upon standard age-specific mortality rates for England and Wales combined.

Overall, only 68 deaths had occurred through 1978, though 72.4 were expected. Twenty-one were due to malignant neoplasms versus 18.6 expected. Deaths from cancer of the stomach were statistically significantly elevated over all age groups (5 observed versus 1.9 expected $P < 0.05$) with deaths to persons in the 55-64 age group contributing the largest portion (3 observed versus 0.7 expected, $P < 0.05$). On the other hand, a statistically significant elevated risk of cancer of the lung, trachea, and bronchus appeared only in the age group 15-44 (3 observed versus 0.7 expected, $P < 0.05$) but not in any other age group.

In a nested (within the larger study) small case control study, the three lung cancer cases were compared with controls (born the same year and living beyond the date of death of the case) with respect to duration of employment. In one case the duration of exposure was significantly greater than the average for the matched controls. In the other two cases, the differences were not significant. The results are equivocal at best.

There are many difficulties with this study, not the least of which is the lack of data regarding actual measured levels of exposure to acrylonitrile during the period 1950 through 1968, according to the author. This lack of quantitative data makes it difficult to distinguish between plants in terms of their relative exposure levels. Implicit in the assumption of lumping the employed populations of six different factories together is that they are all relatively similar with respect to levels of exposure. This is usually not the case, however, for some factories tend to be better controlled than others.

Other problems with this study include the relatively short follow-up in the small subgroup of the cohort where one would expect to have the greatest risk, i.e., the 158 men who had the earliest exposure in 1950-58. This group was followed for a minimum of 20 years resulting in an accumulation of 3,241 person-years of which only 780 person-years occurred to individuals age 55 and over. Like several other studies, this is a relatively youthful cohort and the number of expected deaths is too small to provide confidence in the results. The findings of this study are only suggestive due to small numbers, youthfulness of the cohort, insufficient follow-up, and lack of consideration of effects of smoking. However, this study should be followed several additional years to see if the significant excesses of lung and stomach cancer found in this study remain high.

### 13.5.2.5 Monson (1978)

An epidemiologic study was conducted by Richard R. Monson for the B.F. Goodrich Company and the United Rubber, Cork, Linoleum, and Plastic Workers of America (Federal Register, 1978). This retrospective cancer morbidity and mortality study included some workers with exposure to acrylonitrile. Among workers with potential exposure to acrylonitrile (extent of exposure not known), there was a slight overall excess in the number of deaths from cancer observed compared to the number expected (the data used for determining the expected number were not specified): lung cancer deaths (7 observed versus 4.4 expected); genitourinary cancer deaths (2 observed versus 1.6 expected deaths, and 6 observed versus 3.1 expected incidences); and Hodgkin's disease deaths (2 observed versus 0.3 expected). Deaths from all causes (cancer and noncancer) were not significantly elevated in the worker study group. Because the workers in this study had potential for exposure to other carcinogens, the Occupational Safety and Health Administration concluded that the study could not be used to support an association between acrylonitrile exposure and human cancer development (Federal Register, 1978).

### 13.5.2.6 Zack (1980, unpublished)

Judith A. Zack (1980, unpublished) of the Monsanto Chemical Company investigated the mortality experience of 76 white males at the Texas City facility and 276 white males at the Decatur facility who were exposed to acrylonitrile for a minimum of 6 months prior to January 1, 1968 and followed until December 31, 1977. A total of 15 deaths, all causes, was found to have occurred during that interval of time, 9 at the Texas City facility and 6 at the Decatur facility. Of the 15 deaths, 3 were attributable to cancer, at all sites. Only one was due to respiratory cancer. The author expected 18.11 deaths, all causes, based upon the Monson life table technique with 2.80 malignant neoplasms expected. The author concluded that although the number of deaths is small, the observed numbers do not differ significantly from the expected numbers.

This study is relatively insensitive in its ability to assess a cancer risk in this cohort of acrylonitrile workers for two reasons. First, because of the limited cohort size, the small numbers of expected deaths (18.11 due to all causes, 2.8 due to malignant neoplasms, 0.84 due to respiratory malignancy) do not provide enough information to place any confidence in the statement of nonsignificance made by the author. Second, the usual latent period for cancer of most sites is in excess of 15 years. Although the study design guaranteed at least 10 years of lapsed time since onset of employment to the cutoff date, still 52% or 183 of 352 members of the cohort were observed for no longer than 15 years since initial employment, a time frame which is probably insufficient for a carcinogenic potential to manifest itself, if, in fact, one is present. This report cannot be considered supportive of a "negative" risk assessment of acrylonitrile with respect to its cancer-causing potential in humans.

### 13.5.2.7 Gaffey and Strauss (1981, unpublished)

The Gaffey and Strauss study (1981, unpublished) is a cohort mortality study of 326 white males who were potentially exposed to acrylonitrile in a chemical plant in Decatur, Alabama. This plant was also included in the earlier study by Zack (unpublished). The cohort was defined to be all white males who had achieved 6 months of employment at the plant between April 1952 and December 1953. Follow-up for vital status ascertainment was continued to the end of 1977 and was 95% complete according to the author. However, it was noted that 73 who were found "alive" by retail credit check were subsequently assumed to be "alive" by virtue of the fact that their names appeared in a current telephone directory. Such an assumption may be unwarranted without actual confirmation with the study member. Many widows keep their telephone listing in their husbands' names for security reasons. Another 15 remained with an unknown vital status.

Only 26 deaths were observed during this time frame resulting in an unusually low standard mortality ratio (SMR) overall of 47, while for malignant neoplasms the SMR was only 37. Only 4 of the 26 observed deaths were attributable to cancer. And of these four only two were cancer of the lung (expected = 3.74), while the remaining two were cancer of the kidney (expected = 0.3). Workers in this study could not be classified by level of exposure according to the author. However, the cohort was first dichotomized into hourly versus salaried employees and then into maintenance versus everyone else. No significant excessive risks were evident from the data presented in any of the groups mentioned, not even after a 20-year follow-up. The author notes that the small number of malignancies observed makes any conclusions at best tentative.

Furthermore, the definition of the cohort was not restrictive enough to identify only an "exposed" population for study. The author points out that 21 of the members of his present cohort were also members of the earlier cohort study by Zack. Zack was careful to include only white male employees who "worked in an acrylonitrile manufacturing or processing area" for a minimum of 6 months prior to January 1, 1968). In fact, during the period 1952-53 Zack noted that only 17 employees of the Decatur plant could qualify for inclusion into her cohort of 352 males, while Gaffey counted everyone who was there at start-up (326 white males) including 95 salaried employees. Hence, there is a distinct likelihood that Gaffey's cohort consists of large numbers of unexposed employees. Thus, in addition to the healthy worker effect and incomplete follow-up, it is likely that the relative insensitivity of this analysis to detect a significant risk is due to 1) the relatively youthful nature of the study group (average age at the end of follow-up was only 54.9 years), 2) the inclusion of large numbers of unexposed or minimally exposed employees in the cohort, and 3) the possibility that large numbers of workers were classified erroneously as "live" via the telephone directory check. These problems make this study questionable with respect to the detection of a cancer risk.

### 13.5.2.8 Kiesselbach *et al*. (1980, unpublished)

This study appears to be a cohort mortality study of some 884 male workers who were presumably exposed to acrylonitrile for a minimum of 1 year during the period from 1950 to August 1, 1977 in 16 different plants (pilot, laboratory, and production) of Bayer's Leverkusen Division in West Germany. Workers selected for inclusion into the cohort were involved in the production and processing of acrylonitrile. Follow-up was complete on 93.2% of the cohort. Only 60 persons were without a vital status ascertainment by the close of the study. The male population of the German state of Nordrhein-Westfalen was used as a reference population, although no indication is given that any of the 16 plants are located in that state. A total of 58 persons died in this cohort compared to 104.3 expected. By contrast, deaths due to malignant neoplasms ("Tumors" as they are referred to in the translation) were very close to expected (20 observed versus 20.4 expected). Similarly, 6 respiratory "tumors" were observed versus 6.9 expected. No excessive risks appeared even considering "risk time" after 10 years and 15 years. With respect to lung tumors after a 15 years "risk time," there were 2 observed versus 5.6 expected (assuming "risk time" is the translator's word for follow-up). The authors noted that there were no existing data on acrylonitrile concentration in the air in any work areas over the period of the study.

There are several problems with this study. One of the more important concerns was the selection criteria for inclusion of male workers in the cohort. Originally the authors had in their possession lists containing 1973 names of former and active employees of the company. These lists were submitted to present and former plant managers, supervisors, and foremen to determine the degree to which each person on the list was exposed to acrylonitrile and the periods of time covered by this exposure. To be included each worker must have worked more than 12 months in one of the 16 plants and either worked at least 30% of his time with acrylonitrile, or if less than 30%, he had to have been under a heavy acrylonitrile burden such as would be found in test plants, according to the authors. Over a thousand persons were eventually excluded from the cohort through this review process.

This selection can be affected by recall bias on the part of the reviewers. They are more likely to better remember details regarding the working status and working conditions of actively employed persons than of inactive and former employees. Managers, supervisors, and foremen frequently cannot recall even the names of former employees. Active employees, who have better survivorship, are likely to be overrepresented as a result. The combined total effect of such a biased selection, the influence of the healthy worker effect, and the lack of a complete follow-up, such as occurred in this study, could have led to a substantial underestimation of risk.

Another major concern involves the computation of expected deaths in this study, i.e., the choice of an appropriate comparison population as well as the proper allocation of person-years into the appropriate risk categories as the members of the cohort pass through those categories in time. The greatest deficit of lung cancer deaths compared to expected appeared in members of the cohort who attained an age of 60 and over. With respect to all causes combined, the greatest deficit occurred in persons 45 and over. Only 937 person-years were generated in the age group 60+ in this cohort (out of 13,375 person-years altogether), but the authors calculate that 3.708 lung cancer deaths could have been expected to occur versus only 1 observed death in this age group. This deficit of deaths in persons age 60 and above was reversed in persons under age 60 (5 observed versus 3.23 expected). Such a sudden reversal of risk ratios leads one to suspect that a methodologic problem exists in the analysis. Since the greatest risk of deaths occurs to older persons and is reflected in the death rates based on that age group, either an improper allocation of person-years to older age categories or overestimates of the death rates in the comparison population would have the effect of adding additional expected deaths to that cause and would result in an underestimate of the true risk.

Additionally, a justification is needed for the choice of the Nordrheim-Westfalen state as a comparison population. The location of the 16 factories with respect to the comparison population is not given. Furthermore, without exposure data it is impossible to tell how similar these plants are with respect to the extent of exposure to acrylonitrile. Also, the study cohort was a relatively youthful group even after the close of the study. Only 13% of the total person-years accumulated were in the age category 55 and over, the category in which the greatest increase in lung cancer mortality could be expected to occur. In individuals under age 55, lung cancer is a disease which appears infrequently and a rather large number of person-years accumulating to such persons would be necessary to allow the detection of a significant risk if in fact one exists, and only after a sufficiently lengthy latent period has passed.

Furthermore, inconsistencies in the tabular data presented by the authors cast doubt on the validity of the presented findings. Two examples are 1) the calculation of expected deaths in calendar-time periods where none should appear based on the tabular heading description, and 2) dates of onset of cancer in individuals who exhibit more exposure to acrylonitrile than their ages warrant (one individual was exposed 12 years before he was born). In short, it appears that this study suffers from a general lack of critical review by its author.

Therefore, because of the problems and inconsistencies in this study, the results do not provide evidence to support a lack of a cancer risk to acrylonitrile-exposed employees and cannot be used to refute the results of the O'Berg study as was suggested by the author.

### 13.5.2.9 Herman (1981, unpublished)

A cohort mortality study of 989 male employees in two rubber plants at Baton Rouge and Scotts Bluff, Louisiana was conducted by D. R. Herman (1981, unpublished). There were 799 wage and 190 salaried employees having a minimum of 1 year service in the plants during the period 1951 through 1977. The author reported that race was known on 85.5% of this cohort of 989 males; 162 (24.8%) were black, while 654 (60.7%) were white. Vital status ascertainment was accomplished on a cohort that included 88 females. In this larger cohort 97% were successfully followed to the end of 1977. Only 28 remained with an unknown vital status. The 88 females were excluded from subsequent analyses because of the small number.

Expected deaths were generated separately for wage versus salaried employees as follows: for salaried workers, because almost all were white and a few were unknown with respect to their race, expected deaths were generated utilizing U.S. white male age and calendar-time-specific mortality rates only. However, race was not known for 40% of the wage workers. A separate calculation of expected deaths was done for known whites and blacks by the method described above. In the remaining 40% with unknown race, two calculations of expected deaths were accomplished, first, assuming they were all white, and second, assuming they were all black. The author indicated that these represented high and low estimates. Since the author also knew that about 75% of the total cohort was white, a weighted average of these two separate estimates was obtained for this group as follows:

$$\begin{aligned}\text{Expected-deaths (weighted)} &= 3/4 \times \text{(white expected deaths)}\\ &\quad + 1/4 \times \text{(black expected deaths)}\end{aligned}$$

No further explanation is given.

Overall, 59 observed deaths occurred to the 989 males of the cohort compared to 92 expected based on the above method. Eleven of these were "neoplasms" versus 16.13 expected. Only 3 lung cancers were found versus 5.34 expected. This rather striking shortfall of deaths compared to expected can be attributed only partially to the healthy worker effect and to the fact that no cause of death was found for 4 of the 59 deaths. More importantly, at the close of the study on December 31, 1977, few members of the cohort had reached age 60 and beyond (less than 9%), the age category in which the greatest mortality is likely to occur. In addition, if latent factors are also considered, then it is unlikely that a carcinogenic effect would be seen in this relatively youthful group. Time since onset of employment was not considered, although the author himself suggested another 10 years of surveillance would be required before any exposure-related deaths would occur. No exposure data are provided. This study provides little evidence to support the premise that exposure to acrylonitrile does not increase the risk of cancer.

### 13.5.2.10 Stallard (1982, unpublished)

Stallard (1982, unpublished) conducted a cohort mortality study of 419 white male employees of an oil company facility in Lima, Ohio. The production of acrylonitrile began at this plant March 14, 1960. Members of the cohort had to have been employed at the facility at some time during the period March 14, 1960 to March 14, 1980 in a job that was assessed by plant industrial hygiene personnel as having potential exposure to acrylonitrile. Follow-up through March 14, 1980 was complete on 92% of the cohort (33 remained lost to follow-up) expected deaths were generated through the utilization of 1970 age-cause specific death rates in white males for the United States and separately for the State of Ohio.

Only 7 deaths occurred during this time span versus 25.5 expected based on U.S. rates, another surprising shortage of deaths overall. Of the seven deaths, four were due to malignant neoplasms versus 4.66 expected based on U.S. rates and 4.99 expected based on Ohio rates. Of these four malignant neoplasms, two were due to lung cancer versus 1.56 expected based on U.S. rates. Unfortunately, the expected deaths calculated for lung cancer based on the rates for Ohio are in error (in the age group 45-64, the author shows a figure 15.4 expected deaths; the correct figure is 1.54 and the resulting corrected total number of expected lung cancer deaths all ages combined based on Ohio death rates is 1.78).

Aside from the biases introduced by the incomplete follow-up of all employees (former and present) and the healthy worker effect, a major problem with this study is the relative youthfulness of the cohort even at the end of the observation period (only one person in the successfully followed portion of the cohort of some 386 persons reached age 65). In fact the author himself reported that 87% of the cohort was under age 45 in 1970. Thus, the relative youthfulness of the cohort and a follow-up of insufficient duration did not allow an accurate assessment of the latent effects of the risk of cancer in this cohort. The author himself states the study is "inconclusive" and cannot be used to "support or refute whether or not acrylonitrile is carcinogenic in humans."

### 13.5.3 Quantitative Estimation

This quantitative section deals with the unit risk for acrylonitrile in air and water and the potency of acrylonitrile relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ug/m$^3$ of the agent in the air they breathe or to 1 ug/l in the water they drink. These calculations are done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

#### 13.5.3.1 Procedures for Determination of Unit Risk

The data used for the quantitative estimate are taken from one or both of the following: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses with an incidence determined by the extrapolation model.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time the dominant view of the carcinogenic process involves the concept that most agents that cause cancer also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxin in the diet). There is also some evidence from animal experiments that is consistent with the linear non-threshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large scale $ED_{01}$ study at the National Center for Toxicological Research and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because it has the best, albeit limited, scientific basis of any of the current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper-limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

### 13.5.3.1.1 Animals

### 13.5.3.1.1.1 Description of the Low-Dose Animal Extrapolation Model

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficents $q_i$, $i = 0, 1, 2, \ldots, k$, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79 developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, $q_1^*$, on parameter $q_1$. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and $R/q_1^*$ is a 95% lower confidence limit on the dose producing an extra risk of R. Let $L_0$ be the maximum value of the log-likelihood function. The upper limit, $q_1^*$, is calculated by increasing $q_1$ to a value $q_1^*$ such that when the log-likelihood is remaximized subject to this fixed value, $q_1^*$, for the linear coefficient, the resulting maximum value of the log-likelihood $L_1$ satisfies the equation

$$2\ (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump _et al_. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, $q_1^*$, is taken as an upper-bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment including the control group.

Whenever the multistage model dose not fit the data sufficiently well, data at the highest dose is deleted, and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_iP_i)^2}{N_iP_i\ (1-P_i)}$$

is calculated where $N_i$ is the number of animals in the $i^{th}$ dose group, $X_i$ is the number of animals in the $i^{th}$ dose group with a tumor response, $P_i$ is the probability of a response in the $i^{th}$ dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever $X^2$ is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

### 13.5.3.1.1.2 Selection of Data

For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of the data sets from several studies to use in the model. It may also be appropriate to correct for metabolism differences between species and absorption factors via different routes of administration. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed as follows.

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level, and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, $q_1^*$, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data that has the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of $q_1^*$, estimated from each of these data sets, is used for risk assessment. The geometric mean of numbers $A_1$, $A_2$, ..., $A_m$ is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

### 13.5.3.1.1.3 Calculation of Human Equivalent Dosages from Animal Data

Following the suggestion of Mantel and Schneiderman (1975), we assume that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the two-thirds power of the weight as would be the case for a perfect sphere, the exposure in mg/day per two-thirds power of the weight is also considered to be equivalent exposure. In an animal experiment this equivalent dose is computed in the following manner. Let

$L_e$ = duration of experiment
$l_e$ = duration of exposure
m = average dose per day in mg during administration of the agent (i.e., during $l_e$), and
W = average weight of the experimental animal

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

### 13.5.3.1.1.3.1 Oral

Often exposures are not given in units of mg/day and it becomes necessary to convert the given exposures into mg/day. For example, in most feeding studies, exposure is in terms of ppm in the diet. Similarly, in drinking water studies, exposure is in ppm in the water. In these cases the exposure in mg/day is

$$m = \text{ppm} \times F \times r$$

where ppm is parts per million of the carcinogenic agent in the diet or water, F is the weight of the food or water consumed per day in kg, and r is the absorption fraction. In the absence of any data to the contrary, r is assumed to be equal to one. For a uniform diet, the weight of the food consumed is proportional to the calories required, which in turn is proportional to the surface area or 2/3rds power of the weight. Water demands are also assumed proportional to the surface area, so that

$$m \propto \text{ppm} \times W^{2/3} \times r$$

or

$$\frac{m}{rW^{2/3}} \propto \text{ppm}$$

As a result, ppm in the diet or in water is often assumed to be an equivalent exposure between species. However, this is not justified because the calories/kg of food are very different in the diet of man compared to laboratory animals primarily due to moisture content differences. Consequently, the amount of drinking water required by each species differs also because of the amount of moisture in the food. Therefore, an empirically-derived factor, $f = F/W$, is used which is the fraction of a species body weight that is consumed per day as food or water. We use the following rates:

|  |  | Fraction of Body Weight Consumed as |  |
|---|---|---|---|
| Species | W | $f_{food}$ | $f_{water}$ |
| Man | 70 | 0.028 | 0.029 |
| Rats | 0.35 | 0.05 | 0.078 |
| Mice | 0.03 | 0.13 | 0.17 |

Thus, when the exposure is given as a certain dietary or water concentration in ppm, the exposure in $mg/W^{2/3}$ is

$$\frac{m}{rW^{2/3}} = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}$$

When exposure is given in terms of mg/kg/day = $m/Wr = s$, the conversion is simply

$$\frac{m}{rW^{2/3}} = s \times W^{1/3}$$

### 13.5.3.1.1.3.2 Inhalation

When exposure is via inhalation, the calculation of dose can be considered for two cases where 1) the carcinogenic agent is either a completely water-soluble gas or an aerosol and is absorbed proportionally to the amount of air breathed in, and 2) where the carcinogen is a poorly water-soluble gas which reaches an equilibrium between the air breathed and the body compartments. After equilibrium is reached, the rate of absorption of these agents is expected to be proportional to the metabolic rate, which in turn is proportional to the rate of oxygen consumption, which in turn is a function of surface area.

### 13.5.3.1.1.3.2.1 Case 1

Agents that are in the form of particulate matter or virtually completely absorbed gases, such as sulfur dioxide, can reasonably be expected to be absorbed proportional to the breathing rate. In this case the exposure in mg/day may be expressed as

$$m = I \times v \times r$$

where I = inhalation rate per day in $m^3$, v = $mg/m^3$ of the agent in air, and r = the absorption fraction.

The inhalation rates, I, for various species can be calculated from the observations (FASEB, 1974) that 25 g-mice breathe 34.5 liters/day and 113-g rats breathe 105 liters/day. For mice and rats of other weights, W (in kilograms), the surface area proportionality can be used to find breathing rates in $m^3$/day as follows:

$$\text{For mice, } I = 0.0345\ (W/0.025)^{2/3}\ m^3/\text{day}$$

$$\text{For rats, } I = 0.105\ (W/0.113)^{2/3}\ m^3/\text{day}$$

For humans, the value of 20 $m^3$/day* is adopted as a standard breathing rate (ICRP, 1977).

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*From "Recommendation of the International Commission on Radiological Protection," page 9. The average breathing rate is $10^7$ $cm^3$ per 8-hour workday and $2 \times 10^7$ $cm^3$ in 24 hours.

The equivalent exposure in $mg/W^{2/3}$ for these agents can be derived from the air intake data in a way analogous to the food intake data. The empirical factors for the air intake per kg per day, $i = I/W$, based upon the previously stated relationships, are tabulated as follows:

| Species | W | i = I/W |
|---|---|---|
| Man | 70 | 0.29 |
| Rats | 0.35 | 0.64 |
| Mice | 0.03 | 1.3 |

Therefore, for particulates or completely absorbed gases, the equivalent exposure in $mg/W^{2/3}$ is

$$d = \frac{m}{W^{2/3}} = \frac{Ivr}{W^{2/3}} = \frac{iWvr}{W^{2/3}} = iW^{1/3}vr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the fraction absorbed, r, is assumed to be the same for all species.

### 13.5.3.1.1.3.2.2 Case 2

The dose in mg/day of partially soluble vapors is proportional to the $O_2$ consumption, which in turn is proportional to $W^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the $O_2$ consumption as $O_2 = k\ W^{2/3}$, where k is a constant independent of species, it follows that

$$m = k\ W^{2/3} \times v \times r$$

or

$$d = \frac{m}{W^{2/3}} = kvr$$

As with Case 1, in the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or ug/m$^3$ in experimental animals is equivalent to the same concentration in humans. This is supported by the observation that the minimum alveolar concentration necessary to produce a given "stage" of anesthesia is similar in man and animals (Dripps et al., 1977). When the animals are exposed via the oral route and human exposure is via inhalation or vice-versa, the assumption is made, unless there is pharmacokinetic evidence to the contrary, that absorption is equal by either exposure route.

### 13.5.3.1.1.4 Calculation of the Unit Risk from Animal Studies

The 95% upper-limit risk associated with d mg/kg$^{2/3}$/day is obtained from GLOBAL79 and, for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp-(q_1^*d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value the number of mg/kg$^{2/3}$/day corresponding to one unit of X is determined and substituted into the above relationship. Thus, for example, if X is in units of ug/m$^3$ in the air, then for case 1, $d = 0.29 \times 70^{1/3} \times 10^{-3}$ mg/kg$^{2/3}$/day, and for case 2, d = 1, when ug/m$^3$ is the unit used to compute parameters in animal experiments.

Exposures given in terms of ppm in air can be converted to units of mg/m$^3$ by the formula

$$1 \text{ ppm} = 1.2 \times \frac{\text{molecular weight (gas) mg/m}^3}{\text{molecular weight (air)}}$$

Note that an equivalent method of calculating unit risk would be to use mg/kg for the animal exposures and then increase the jth polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1, 2, \ldots, k$$

and use mg/kg equivalents for the unit risk values.

### 13.5.3.1.1.4.1 Adjustment for Less Than Lifespan Duration of Experiment

If the duration of experiment, $L_e$, is less than the natural lifespan of the test animal, L, the slope, $q_1^*$, or more generally the exponent, g(d), is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose, d, is continued, the age-specific rate of cancer will continue to increase as a constant function of the background rate. The age-specific rates for humans increase at least by the 2nd power of the age and often by a considerably higher power as demonstrated by Doll (1971). Thus, we would expect the cumulative tumor rate to increase by at least the 3rd power of age. Using this fact, we assume that the slope, $q_1^*$, or more generally the exponent, g(d), would also increase by at least the 3rd power of age. As a result, if the slope $q_1^*$ [or g(d)] is calculated at age $L_e$, we would expect that if the experiment had been continued for the full lifespan, L, at the given average exposure, the slope $q_1^*$ [or g(d)] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Crump (1979) where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp [-f(t) \times g(d)]$$

### 13.5.3.1.1.5 Interpretation of Quantitative Estimates

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure, preferably by inhalation.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper-limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as an accurate representation of the true cancer risks even when the exposures are accurately defined. The estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

### 13.5.3.1.1.6 Alternative Methodological Approaches

The methods used by the CAG for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism in this respect is the linear non-threshold extrapolation model. There are a variety of other extrapolation models that could be used, all of which would give lower risk estimates. These alternative models have not been used by the CAG in the following analysis but are included for comparison in the appendix. The models presented there are the one-hit, Probit, and Weibull. The CAG feels that with the limited data available from these animal bioassays, especially at the high-dose levels required for testing, almost nothing is known about the true shape of the dose-response curve at low environmental levels. The position is taken by the CAG that the risk estimates obtained by use of the linear non-threshold model are plausible upper-limits and the true risk could be lower.

In terms of the choice of animal bioassay as the basis for extrapolation, the general approach is to use the most sensitive responder on the assumption that humans are as sensitive as the most sensitive animal species tested. For acrylonitrile, the average response of all of the adequately tested bioassay animals was used; this is because three well-conducted valid drinking water studies using different strains of rats showed similar target organs and about the same level of response.

Extrapolations from animals to humans could also be done on the basis of relative weights rather than relative surface areas. The latter approach, used here, has more basis in human pharmacological responses; it is not clear which of the two approaches is more appropriate for carcinogens. In the absence of information on this point, it seems appropriate to use the most generally obtained method, which also is more conservative. In the case of the acrylonitrile drinking water studies, the use of extrapolation based on surface area rather than weights increases the unit risk estimates by a factor of 5.8.

### 13.5.3.1.2 Humans--Model for Estimation of Unit Risk Based on Human Data

If human epidemiologic studies and sufficiently valid exposure information are available for the compound, they are always used in some way. If they show a carcinogenic effect, the data are analyzed to give an estimate of the linear dependence of cancer rates on lifetime average dose, which is equivalent to the factor $B_H$. If they show no carcinogenic effect when positive animal evidence is available, then it is assumed that a risk does exist, but it is smaller than could have been observed in the epidemiologic study, and an upper-limit to the cancer incidence is calculated assuming hypothetically that the true incidence is just below the level of detection in the cohort studied, which is determined largely by the cohort size. Whenever possible, human data are used in preference to animal bioassay data.

Very little information exists that can be utilized to extrapolate from high exposure occupational studies to low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, the response is measured in terms of the relative risk of the exposed cohort of individuals compared to the control group. The mathematical model employed assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), $P_0$, may be represented by the linear equation

$$P_0 = A + B_H x$$

where A is the lifetime probability in the absence of the agent, and x is the average lifetime exposure to environmental levels in some units, say ppm. The factor $B_H$ is the increased probability of cancer associated with each unit increase of r, the agent in air.

If we make the assumption that R, the relative risk of lung cancer for exposed workers compared to the general population, is independent of the length or age of exposure but depends only upon the average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H (x_1 + x_2)}{A + B_H x_1}$$

or

$$RP_0 = A + B_H (x_1 + x_2)$$

where $x_1$ = lifetime average daily exposure to the agent for the general population, $x_2$ = lifetime average daily exposure to the agent in the occupational setting, and $P_0$ = lifetime probability of dying of cancer with no or negligible acrylonitrile exposure.

Substituting $P_0 = A + B_H x_1$ and rearranging gives

$$B_H = P_0 (R - 1)/x_2$$

To use this model, estimates of R and $x_2$ must be obtained from the epidemiologic studies. The value $P_0$ is derived from the age-cause-specific death rates for combined males found in the 1976 U.S. Vital Statistics tables (U.S. Department of Health and Education, and Welfare, 1976) using the life table methodology. For lung cancer the estimate of $P_0$ is 0.036. This methodology is used in the section on unit risk based on human studies.

### 13.5.3.2 Acrylonitrile Risk Estimates

#### 13.5.3.2.1 Unit Risk Estimate Based on Human Studies

Of the ten epidemiologic studies reviewed in this document, the study of workers at the Du Pont May Plant, Camden, South Carolina (O'Berg, 1980), clearly presented the most significant evidence of acrylonitrile as a human lung carcinogen. Furthermore, it is the only plant for which we have been able to estimate exposure levels. Therefore, this study is used to estimate a unit risk for human inhalation. Furthermore, the lack of exposure levels for the other studies precludes estimating an upper-limit risk based on negative studies.

The carcinogenic effectiveness (potency) of acrylonitrile, $B_H$, is calculated as follows:

$$B_H = \frac{P_0 \ (R - 1)}{X_2}$$

where $P_0 = 0.036$, the background lifetime probability of death due to respiratory cancer. This factor is derived from the 1976 U.S. Vital Statistics tables via a life table calculation (CAG, 1978).

The Du Pont Chemical Company follow-up study by O'Berg (1980) of 1,345 workers exposed to acrylonitrile between 1950 and 1966 found the observed and expected number of cancer cases to be 13 versus 5.5, respectively, in workers with at least moderate exposure and with a probable latency period of at least 15 years. This excess incidence was found to be statistically significant ($P < 0.05$). Half of this excess was due to respiratory cancer, 5 versus 1.4, which is also statistically significant ($P < 0.05$). If we adjust for the effects of smoking as described earlier, we have 5 observed vs. 1.6 expected, which is statistically significant. Therefore, the relative risk, R, is 5.0/1.6 = 3.1. For this analysis we will equate respiratory cancer incidence with mortality.

Estimates of exposure at the plant are those suggested by J. Brown, an industrial hygienist at NIOSH, who visited the plant in 1978. She states, "While no statistical data were used to establish the system used (to rank the included cohort according to exposure), the Du Pont representatives agreed that 8-hour time-weighted averages might be used to represent possible past exposure levels, based principally on past recall of process levels taken some years past" (NIOSH, 1978). Also in the Brown report were some measurements of potential acrylonitrile exposure which averaged between 1 and 3 ppm for the first three quarters of 1977 and less than 1 ppm for the last quarter.

Since the workers were exposed to at least a moderate level (10 ppm = moderate level, 20 ppm = high level), we will assume 15 ppm to be the 8-hour time-weighted average (TWA). We estimate the uncertainty attached to this estimate to be as high as a factor of 5.

To convert the 8-hour TWA exposure to a lifetime average, we use

$$X_1 = 15 \text{ ppm} \times \frac{8}{24} \times \frac{240}{365} \times \frac{9}{60}$$

$$= 0.5 \text{ ppm continuous equivalent lifetime exposure}$$

where 9 years is estimated to be the average exposure duration, and 60 years is estimated to be the maximum possible age at the end of the observation period.

The value of $B_H$ [in $(\text{ppb})^{-1}$] is derived using the above estimates as follows:

$$B_H = \frac{0.036\ (3.1 - 1)}{500 \text{ ppb}}$$

$$= 1.5 \times 10^{-4}\ (\text{ppb})^{-1}$$

Therefore, the lifetime risk of cancer for people continuously exposed to 1 ppb is $1.5 \times 10^{-4}$.

To express risk in terms of $ug/m^3$ concentration, the conversion factor for acrylonitrile is

$$1 \text{ ppb} = 1.2 \times \frac{\text{MW acrylonitrile}}{\text{MW air}} = \frac{1.2(53.06)}{28.8} = 2.21 \text{ ug/m}^3$$

or

$$1 \text{ ug/m}^3 = 0.45 \text{ ppb}$$

Therefore the upper-bound risk associated with a lifetime exposure of 1 $ug/m^3$ in air is

$$P = 1.5 \times 10^{-4} \times 0.45 = 6.8 \times 10^{-5}$$

#### 13.5.3.2.2 Unit Risk Estimate Based on Animal Studies

Unit risk estimates are calculated based on both inhalation and drinking water studies.

##### 13.5.3.2.2.1 Drinking Water Studies

The three rat bioassays of acrylonitrile in drinking water have all shown significant increases in brain and/or spinal cord astrocytomas, Zymbal gland carcinomas, and stomach papillomas/carcinomas (Tables 13-21 through 13-24). These three studies are evaluated separately and then combined to determine the mean estimate.

The individual results of the Dow Chemical Company rat study (Quast et al., 1980a) are shown in Table 13-21 and 13-22. Since several tumor sites are affected, the overall risk of tumors is determined from the number of animals having tumors that are statistically significant at any of the sites. This is shown in Table 13-35A. Presented there is the percentage of animals by sex and by dose with the tumors discussed above. In addition, the small intestine and

## TABLE 13-35. NUMBER (PERCENTAGE) OF RATS DEVELOPING TUMORS IN AT LEAST ONE TARGET ORGAN: THREE ACRYLONITRILE DRINKING WATER BIOASSAYS, BY DOSE AND BY SEX

### A. Dow Chemical Company (Quast *et al.*, 1980a)
(Target Organs in Tables 13-21 and 13-22)

| Males | | Females | | |
|---|---|---|---|---|
| Animal Dose (mg/kg/day) | Number/Total (Percent) | Animal Dose (mg/kg/day) | Including Mammary Gland Tumors Number/Total (Percent) | Excluding Mammary Gland Tumors Number/Total (Percent) |
| 0 | 4/80 (5.0%) | 0 | 59/80 (73.8%) | 3/80 (3.8%) |
| 3.42 | 18/47 (38.3%)† | 4.36 | 47/48 (97.9%)§ | 24/48 (50.0%)† |
| 8.53 | 36/48 (75.0%)† | 10.76 | 46/48 (95.8%)§ | 37/48 (77.1%)† |
| 21.18 | 45/48 (93.8%)† | 4.27 | 48/48 (100%)§ | 45/48 (93.8%)† |

### B. Bio/Dynamics Inc. (1980a)
(Target Organs in Table 13-23)

| Males | | Females | |
|---|---|---|---|
| Animal Dose (mg/kg/day) | Number/Total (Percent) | Animal Dose (mg/kg/day) | Number/Total (Percent) |
| 0 | 6/100 (6.0%) | 0 | 1/100 (1.0%) |
| 0.09 | 6/98 (6.1%) | 0.15 | 5/100 (5.0%) |
| 7.98 | 36/98 (36.7%) | 10.70 | 41/99 (41.4%) |

C. Bio/Dynamics Inc. (1980b)
(Target Organs in Table 13-24)

| Males | | Females | |
|---|---|---|---|
| Animal Dose (mg/kg/day) | Number/Total (Percent) | Animal Dose (mg/kg/day) | Number/Total (Percent) |
| 0 | 5/200 (2.5%) | 0 | 2/199 (1.0%) |
| 0.11 | 4/100 (4.0%) | 0.12 | 2/100 (2.0%) |
| 0.25 | 5/100 (5.0%) | 0.36 | 6/101 (5.9%) |
| 0.81 | 7/100 (7.0%) | 0.82 | 10/97 (10.3%) |
| 2.49 | 20/100 (20.0%) | 3.65 | 13/100 (13.0%) |
| 8.15 | 34/100 (34.0%) | 10.89 | 33/98 (33.7%) |

mammary gland, which are target sites for the female Sprague-Dawley rats, are included. For the mammary gland, however, because the control group incidence is so high, the percentages for the females are tabulated separately, and the risk extrapolation is calculated excluding mammary gland tumors.

To convert the animal doses into a human equivalent dose, the standard approach is to equate the doses on the basis of mg per body surface area. Estimating the weight of the rats to be about 350 grams, the human dosage equivalent to 3.42 mg/kg/day for the male rat (Table 13-35A) is

$$3.42 \text{ mg/kg/day} \div (70/0.350)^{1/3} = 0.6 \text{ mg/kg/day}$$

When these human dose equivalents are used with the animal response data in Table 13-35A, the multistage model yields a value of $q_1^* = 9.9 \times 10^{-1}$ $(\text{mg/kg/day})^{-1}$ for the males, and $q_1^* = 9.2 \times 10^{-1}$ $(\text{mg/kg/day})^{-1}$ for the females excluding mammary gland tumors (Table 13-36). (If the human dose had been assumed equivalent on a mg/kg/day basis, the value of these parameters would be smaller by a factor of $(70/0.35)^{1/3} = 5.8$.)

The results of the two Bio/Dynamics Inc. studies using Sprague-Dawley (1980a) and Fischer 344 (1980b) rats are summarized in Table 13-35B and 13-35C, based on tumors of the individual target organs in Tables 13-23 and 13-24, respectively. The dose levels in Tables 13-35B and 13-35C are presented in mg/kg/day. This allows for a direct comparison between animal studies and an estimate of human dose equivalents. Dividing this animal dose by the factor 5.8 (presented above) yields the human dose equivalent used in the linearized multistage model. When these doses are used with the animal response data, the multistage model yields values for the upper-limit estimates of slope all in the range of 0.3 to 1 $(\text{mg/kg/day})^{-1}$. The individual and combined upper-limit slopes are presented in Table 13-36. The geometric average for males is

## TABLE 13-36. ESTIMATES OF 95% UPPER-LIMIT SLOPES FOR THREE DRINKING WATER STUDIES, BY SEX

| Study | Sex<br>Males | <br>Females |
|---|---|---|
| | $q_1^*$ in $(mg/kg/day)^{-1}$ | |
| Dow Chemical Co. | $9.9 \times 10^{-1}$ | $9.2 \times 10^{-1}$ |
| Bio/Dynamics Inc., 1980a | $4.0 \times 10^{-1}$ | $3.7 \times 10^{-1}$ |
| Bio/Dynamics Inc., 1980b | $4.0 \times 10^{-1}$ | $2.9 \times 10^{-1}$ |
| Geometric Average | $5.4 \times 10^{-1}$ | $4.6 \times 10^{-1}$ |

$5.4 \times 10^{-1}$ (mg/kg/day)$^{-1}$ and $4.6 \times 10^{-1}$ (mg/kg/day)$^{-1}$ for females. The former slightly higher value is chosen as the slope for extrapolation purposes.

In order to estimate the unit risk for 1 ug/liter of acrylonitrile in drinking water, we assume that the average 70 kg person drinks 2 liters of water per day. This corresponds to a daily dose of 2 ug/day x $10^{-3}$ mg/ug x 1/70 kg = $2.86 \times 10^{-5}$ mg/kg/day. The upper-limit unit risk corresponding to 1 ug/liter acrylonitrile concentration in water is then

$$\begin{aligned} P &= 1 - e^{-5.4 \times 10^{-1} \times 2.86 \times 10^{-5}} \\ &= 1.5 \times 10^{-5} \end{aligned}$$

The drinking water study could also be used to estimate the inhalation risk, although such an estimate is expected to be unreliable because of the difference in exposure routes. The following is done for comparison purposes only. The dose rate, d(mg/kg/day), resulting from breathing 20 $m^3$/day of air containing a concentration of 1 ug/$m^3$ can be determined if one assumes that 100% of the inhaled acrylonitrile is absorbed into the body. This was shown to be the case by Young et al. (1977). With this assumption, the dose rate is

$$\begin{aligned} d &= 1\ \text{ug/m}^3 \times 20\ \text{m}^3\text{/day} \times 10^{-3}\ \text{mg/ug} \times 1/70\ \text{kg} \\ &= 2.86 \times 10^{-4}\ \text{mg/kg/day} \end{aligned}$$

The upper-limit estimate of the air unit risk for 1 ug/$m^3$, P, can be found using this value of d and the value of $q_1^*$ estimated above as follows:

$$\begin{aligned} P &= 1 - e^{-5.4 \times 10^{-1} \times 2.86 \times 10^{-4}} \\ &= 1.5 \times 10^{-4} \end{aligned}$$

This is a factor of 2 greater than that derived from the human epidemiologic studies and one order of magnitude greater than that derived from the animal inhalation studies in the following section.

### 13.5.3.2.2.2 Inhalation Studies

Of the two inhalation bioassays previously discussed, the Quast et al. (1980b) study produced a clear carcinogenic effect, while the Maltoni et al. (1977) study produced no statistically significant increases in tumors in rats exposed to low dosages for 12 months. Therefore, the Quast et al. study is used for determining a unit risk estimate via the inhalation route.

The tumor incidence data on individual tumor types have been presented previously (Table 13-32). For the males the response occurs at the following four target organs: Zymbal gland, small intestine, brain, and/or spinal cord. For males the percentages of rats with tumors at one or more of the target organ sites are 5% (5/100), 9% (9/100), and 47% (47/100) for the 0 ppm, 20 ppm, and 80 ppm groups, respectively. For the females the corresponding percentages are 0%, (0/100), 9% (9/100), and 31% (31/100). In analyzing these data, the responses of both sexes were fit by the model. The estimate of carcinogenic potency for the females was slightly higher and is presented below.

In this study, animals were exposed to 0, 20, or 80 ppm of acrylonitrile 6 hours per day, 5 days per week, for 2 years. Therefore, the lifetime average concentration for the 20 ppm group is

$$20 \times \frac{6}{24} \times \frac{5}{7} = 3.57 \text{ ppm}$$

Similarly, the lifetime equivalent dosage for the 80 ppm group is 14.29 ppm. Using the linearized multistage model with incidence data for females, the carcinogenic potency is $q_1^* = 3.35 \times 10^{-2}\ (\text{ppm})^{-1}$, and the upper-bound estimate of the lifetime risk of cancer associated with 1 ug/m$^3$ = $4.53 \times 10^{-4}$ ppm of acrylonitrile is

$$P = 1 - \exp(-3.35 \times 10^{-2} \times 4.53 \times 10^{-4})$$
$$= 1.5 \times 10^{-5}$$

### 13.5.3.2.3 Summary of Unit Risks

In summary, the upper-limit unit risk estimates for humans breathing 1 ug/m$^3$ of acrylonitrile in ambient air (equivalent to 0.45 ppb) are $6.8 \times 10^{-5}$ based on the occupational study, $1.5 \times 10^{-4}$ based on the rat drinking water study, and $1.5 \times 10^{-5}$ based on the rat inhalation study. Parenthetically, it should be noted that if the human equivalent dose assumption were changed to dose per body weight, the unit risk for inhalation based on the rat drinking water study would be $1.5 \times 10^{-4} \times 1/5.8 = 2.6 \times 10^{-5}$, a value which is between the other two estimates. Although this estimate is considered unreliable because of the inappropriate route of administration, it is included here as a matter of interest. The upper-limit unit risk for 1 ug/liter of acrylonitrile in drinking water is estimated to be $1.5 \times 10^{-5}$.

### 13.5.3.3 Relative Potency

One of the uses of unit risk is to compare the potency of carcinogens. To estimate the relative potency on a per mole basis, the unit risk slope factor is multiplied by the molecular weight and the resulting number expressed in terms of (mMol/kg/day)$^{-1}$. This is called the relative potency index.

Figure 13-7 is a histogram representing the frequency distribution of potency indices of 54 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 13-37. When human data are available for a compound, they have been used to calculate the index. When no human data are available, animal oral studies and animal inhalation studies have been used in that order. Animal oral studies are selected over animal inhalation studies because most of the chemicals have animal oral studies; this allows potency comparisons by route.

| 4th quartile | 3rd quartile | 2nd quartile | 1st quartile |
|---|---|---|---|

$1 \times 10^{+1}$ $\quad 4 \times 10^{+2}$ $\quad 2 \times 10^{+3}$

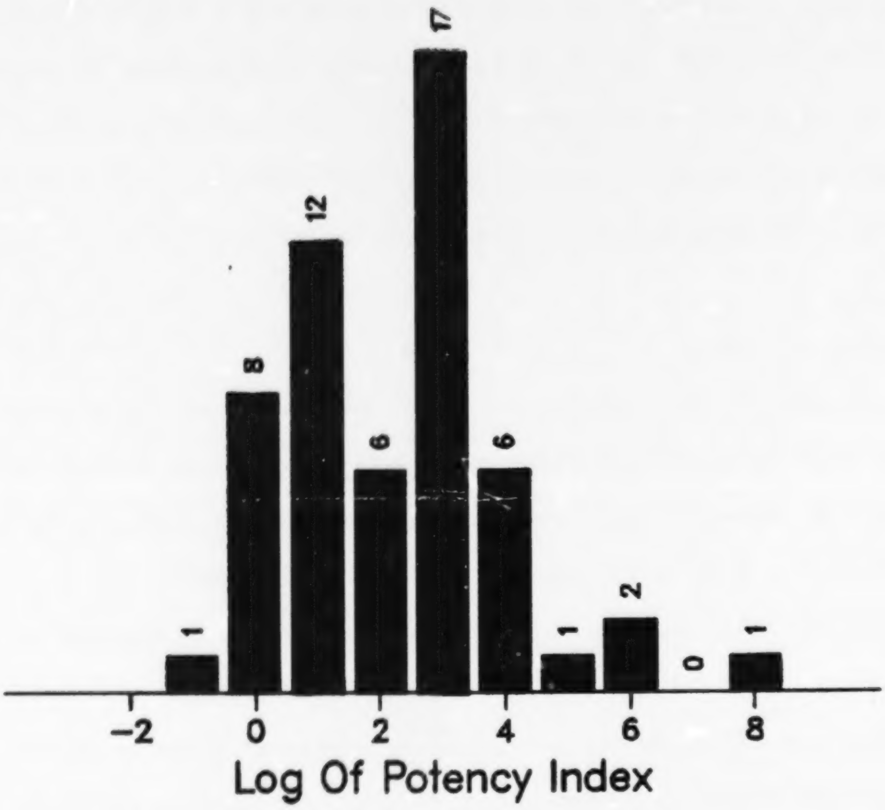

Figure 13-7. Histogram representing the frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 13-37. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS[1,2,3]

| Compounds | Slope $(mg/kg/day)^{-1}$ | Molecular Weight | Potency Index | Order of Magnitude ($log_{10}$ Index) |
|---|---|---|---|---|
| Acrylonitrile | 0.24(W) | 53.1 | $1x10^{+1}$ | +1 |
| Aflatoxin $B_1$ | 2924 | 312.3 | $9x10^{+5}$ | +6 |
| Aldrin | 11.4 | 369.4 | $4x10^{+3}$ | +4 |
| Allyl Chloride | $1.19x10^{-2}$ | 76.5 | $9x10^{-1}$ | 0 |
| Arsenic | 15(H) | 149.8 | $2x10^{+3}$ | +3 |
| B[a]P | 11.5 | 252.3 | $3x10^{+3}$ | +3 |
| Benzene | $5.2x10^{-2}$(W) | 78 | $4x10^{0}$ | +1 |
| Benzidine | 234(W) | 184.2 | $4x10^{+4}$ | +5 |
| Beryllium | 4.86 | 9 | $4x10^{+1}$ | +2 |
| Cadmium | 6.65(W) | 112.4 | $7x10^{+2}$ | +3 |
| Carbon Tetrachloride | $1.30x10^{-1}$ | 153.8 | $2x10^{+1}$ | +1 |
| Chlordane | 1.61 | 409.8 | $7x10^{+2}$ | +3 |
| Chlorinated Ethanes | | | | |
| 1,2-dichloroethane | $6.9x10^{-2}$ | 98.9 | $7x10^{0}$ | +1 |
| hexachloroethane | $1.42x10^{-2}$ | 236.7 | $3x10^{0}$ | 0 |
| 1,1,2,2-tetrachloroethane | 0.20 | 167.9 | $3x10^{+1}$ | +1 |
| 1,1,1-trichloroethane | $1.6x10^{-3}$ | 133.4 | $2x10^{-1}$ | -1 |
| 1,1,2-trichloroethane | $5.73x10^{-2}$ | 133.4 | $8x10^{0}$ | +1 |
| Chloroform | $7x10^{-2}$ | 119.4 | $8x10^{0}$ | +1 |
| Chromium (W) | 41 | 104 | $4x10^{+3}$ | +4 |
| DDT | 8.42 | 354.5 | $3x10^{+3}$ | +3 |
| Dichlorobenzidine | 1.69 | 253.1 | $4x10^{+2}$ | +3 |
| 1,1-dichloroethylene | $1.47x10^{-1}$(I) | 97 | $1x10^{+1}$ | +1 |
| Dieldrin | 30.4 | 380.9 | $1x10^{+4}$ | +4 |

(continued on the following page)

TABLE 13-37. (continued)

| Compounds | Slope (mg/kg/day)$^{-1}$ | Molecular Weight | Potency Index | Order of Magnitude ($\log_{10}$ Index) |
|---|---|---|---|---|
| Dinitrotoluene | 0.31 | 182 | $6\times10^{+1}$ | +2 |
| Diphenylhydrazine | 0.77 | 180 | $1\times10^{+2}$ | +2 |
| Epichlorohydrin | $9.9\times10^{-3}$ | 92.5 | $9\times10^{-1}$ | 0 |
| Bis(2-chloroethyl)ether | 1.14 | 143 | $2\times10^{+2}$ | +2 |
| Bis(chloromethyl)ether | 9300(I) | 115 | $1\times10^{+6}$ | +6 |
| Ethylene Dibromide (EDB) | 8.51 | 187.9 | $2\times10^{+3}$ | +3 |
| Ethylene Oxide | 0.63(I) | 44.0 | $3\times10^{+1}$ | +1 |
| Formaldehyde | $2.14\times10^{-2}$(I) | 30 | $6\times10^{-1}$ | 0 |
| Heptachlor | 3.37 | 373.3 | $1\times10^{+3}$ | +3 |
| Hexachlorobenzene | 1.67 | 284.4 | $5\times10^{+2}$ | +3 |
| Hexachlorobutadiene | $7.75\times10^{-2}$ | 261 | $2\times10^{+1}$ | +1 |
| Hexachlorocyclohexane | | | | |
| technical grade | 4.75 | 290.9 | $1\times10^{+3}$ | +3 |
| alpha isomer | 11.12 | 290.9 | $3\times10^{+3}$ | +3 |
| beta isomer | 1.84 | 290.9 | $5\times10^{+2}$ | +3 |
| gamma isomer | 1.33 | 290.9 | $4\times10^{+2}$ | +3 |
| Methylene Chloride | $7.8\times10^{-3}$ | 84.9 | $7\times10^{-1}$ | 0 |
| Nickel | 1.15(W) | 58.7 | $7\times10^{+1}$ | +2 |
| Nitrosamines | | | | |
| Dimethylnitrosamine | 25.9(not by $q_1^*$) | 74.1 | $2\times10^{+3}$ | +3 |
| Diethylnitrosamine | 43.5(not by $q_1^*$) | 102.1 | $4\times10^{+3}$ | +4 |
| Dibutylnitrosamine | 5.43 | 158.2 | $9\times10^{+2}$ | +3 |
| N-nitrosopyrrolidine | 2.13 | 100.2 | $2\times10^{+2}$ | +2 |
| N-nitroso-N-ethylurea | 32.9 | 117.1 | $4\times10^{+3}$ | +4 |
| N-nitroso-N-methylurea | 302.6 | 103.1 | $3\times10^{+4}$ | +4 |
| N-nitroso-diphenylamine | $4.92\times10^{-3}$ | 198 | $1\times10^{0}$ | 0 |
| PCBs | 4.34 | 324 | $1\times10^{+3}$ | +3 |

(continued on the following page)

TABLE 13-37. (continued)

| Compounds | Slope $(mg/kg/day)^{-1}$ | Molecular Weight | Potency Index | Order of Magnitude ($log_{10}$ Index) |
|---|---|---|---|---|
| Phenols<br>2,4,6-trichlorophenol | $1.99 \times 10^{-2}$ | 197.4 | $4 \times 10^{0}$ | +1 |
| Tetrachlorodioxin | $4.25 \times 10^{5}$ | 322 | $1 \times 10^{+8}$ | +8 |
| Tetrachloroethylene | $3.5 \times 10^{-2}$ | 165.8 | $6 \times 10^{0}$ | +1 |
| Toxaphene | 1.13 | 414 | $5 \times 10^{+2}$ | +3 |
| Trichloroethylene | $1.26 \times 10^{-2}$ | 131.4 | $2 \times 10^{0}$ | 0 |
| Vinyl Chloride | $1.75 \times 10^{-2}$(I) | 62.5 | $1 \times 10^{0}$ | 0 |

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

2. The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

The potency index for acrylonitrile based on the O'Berg study of Dupont workers is $1.3 \times 10^{+1}$ $(\text{mMol/kg/day})^{-1}$. This is derived as follows. The slope estimate from the O'Berg study, $6.8 \times 10^{-5}(\text{ug/m}^3)^{-1}$, is first converted to units of $(\text{mg/kg/day})^{-1}$, assuming a breathing rate of 20 $m^3$ of air per day and a 70 kg person.

$$6.8 \times 10^{-5}\ (\text{ug/m}^3)^{-1} \times \frac{1\ \text{day}}{20\ \text{m}^3} \times \frac{1\ \text{ug}}{10^{-3}\ \text{mg}} \times 70\ \text{kg}$$

$$= 0.24\ (\text{mg/kg/day})^{-1}$$

Multiplying by the molecular weight of 53.1 gives a potency index of $1.3 \times 10^{+1}$. Rounding off to the nearest order of magnitude gives a value of $10^{+1}$, which is the scale presented on the horizontal axis of Figure 13-7. The index of $1.3 \times 10^{+1}$ lies at the bottom of the third quartile of the 54 suspect carcinogens.

Ranking of the relative potency indices is subject to the uncertainty of comparing estimates of potency of different chemicals based on different routes of exposure to different species using studies of different quality. Furthermore, all the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. Thus, these indices are not valid to compare potencies in the experimental or observational range if linearity does not exist there.

## 13.5.4 SUMMARY

### 13.5.4.1 Qualitative Assessment

Acrylonitrile is not a direct-acting carcinogen, and hence the localization and nature of the effects depend on its metabolism. It is probable that the proximal carcinogen is 2-cyanoethylene oxide, since it has been demonstrated as a reaction product with calf thymus DNA. However, the metabolite has not been tested directly for its carcinogenicity. It has been shown to be produced in the liver and possibly circulates to other organs. However, studies have not been done to determine where else in the body this metabolite is produced. There appears to be a clear difference between animals and humans in their tumorigenic response to acrylonitrile: no lung tumors have been produced in animals, and no brain tumors have been observed in humans. There are no human studies on the matabolism of acrylonitrile, and there are no pharmacokinetic studies that would be relevant to the characterization of dose-response relationships at low levels of exposure.

The carcinogenicity of acrylonitrile has been studied in seven cancer bioassays in rats: four in drinking water, one by gastric intubation, and two by inhalation. These are summarized in Table 13-38. In addition, ten epidemiologic studies of cancer incidence have been reported. These are summarized in Table 13-39. A short description of these studies follows.

Quast _et_ _al_. (1980a) administered acrylonitrile in drinking water to Sprague-Dawley rats for 2 years at dose levels of 35, 100, and 300 ppm. A statistically significant incidence of tumors was observed in the central nervous system, Zymbal gland, stomach, tongue, and small intestine in both male and female rats, as well as in the mammary gland of female rats. The occurrence of central nervous system and Zymbal gland tumors in Sprague-Dawley rats was further confirmed in four other studies: a three-generation

## TABLE 13-38. CARCINOGENICITY OF ACRYLONITRILE IN RATS

| Reference | Strain of Rat | Route of Administration | Dose Level* | Treatment Schedule | Site of Tumors |
|---|---|---|---|---|---|
| Quast *et al*. (1980a) | Sprague-Dawley | ingestion in drinking water | 35-300 ppm | 24 mo. | Brain (astrocytomas)<br>Ear canal (Zymbal gland)<br>Stomach (nonglandular portion)<br>Mammary gland (females only)<br>Tongue<br>Pituitary gland<br>Pancreas (males only)<br>Uterus (females only) |
| Beliles *et al*. (1980) | Charles River | ingestion in drinking water | 500 ppm | 45 wk.† | Brain (astrocytomas)<br>Ear canal (Zymbal gland) |
| Bio/Dynamics Inc. (1980a) | Spartan | ingestion in drinking water | 100 ppm | 19-20 mo. | Brain (astrocytomas)<br>Ear canal (Zymbal gland)<br>Stomach |
| Bio/Dynamics Inc. (1980b) | Fischer 344 | ingestion in drinking water | 10-100 ppm | 23-26 mo. | Brain (astrocytomas)<br>Ear canal (Zymbal gland)<br>Stomach<br>Mammary gland (females only) |
| Bio/Dynamics Inc. (1980c) | Spartan | gavage | 10 mg/kg/day | 19-20 mo. | Brain (astrocytomas)<br>Ear canal (Zymbal gland)<br>Stomach<br>Mammary gland (females only) |

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*Not all the tumors indicated for each study were observed at the lower dose.

†This was a three-generation reproductive study; the tumors were observed in the second generation.

(continued on the following page)

TABLE 13-38. (continued)

| Reference | Strain of Rat | Route of Administration | Dose Level* | Treatment Schedule | Site of Tumors |
|---|---|---|---|---|---|
| Maltoni _et_ _al_. (1977) | Sprague-Dawley | inhalation | 5-40 ppm | 4 hr/day<br>5 days/wk.<br>for 12 mo. | Stomach (males only)<br>Mammary gland (females only)<br>Skin (females only) |
| Quast _et_ _al_. (1980b) | Sprague-Dawley | inhalation | 20-80 ppm | 6 hr/day<br>5 days/wk<br>for 24 mo. | Central nervous system<br>Ear canal (Zymbal gland)<br>Gastrointestinal tract (males only); mammary gland (females only); tongue (males only) |

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*Not all the tumors indicated for each study were observed at the lower dose.

†This was a three-generation reproductive study; the tumors were observed in the second generation.

TABLE 13-39. EPIDEMIOLOGIC STUDIES REVIEWED IN ACRYLONITRILE RISK ASSESSMENT

| Study | Results | Observed/Expected | Highlights and Deficiencies |
|---|---|---|---|
| O'Berg (1980) | Positive<br>lung cancer | 5/1.4 ($P < 0.05$) | Significant excess occurred in group with highest exposure followed for the longest time. Excess remains after adjustment for smoking. |
| Delzell and Monson (1982) | Positive<br>lung cancer | 4/1.4 ($P < 0.05$) | Potential exposure to other carcinogens. |
| Thiess _et al._ (1980) | Positive<br>lung cancer<br>lymph cancer | 9/4.4 ($P < 0.05$)<br>4/1.4 ($P < 0.05$) | Incomplete follow-up on foreign workers. Mixed exposures to beta-naphthalamine, polycyclic hydrocarbons, and vinyl chloride. |
| Werner and Carter (1981) | Positive<br>lung cancer<br>stomach cancer | 3/0.7 ($P < 0.05$)<br>5/1.9 ($P < 0.05$) | Relatively short follow-up. Combined Population from six polymerization factories. |
| Monson (1978) | Inconclusive | | Potential exposure to other carcinogens. |
| Zack (1980, unpublished) | Inconclusive | | Small cohort size. Short follow-up on most members of cohort. |
| Gaffey and Strauss (1981, unpublished) | Inconclusive | | Small cohort size. Includes large numbers of minimally or unexposed employees. |

(continued on the following page)

TABLE 13-39. (continued)

| Study | Results | Observed/Expected | Highlights and Deficiencies |
|---|---|---|---|
| Kiesselbach _et al_. (1980, unpublished) | Inconclusive | | Markedly reduced mortality due to 1) healthy worker effect, and 2) possible selection bias in determination of study population. Lack of critical editorial review. |
| Herman (1981, unpublished) | Inconclusive | | Relatively short duration of employment. Insufficient follow-up. |
| Stallard (1982, unpublished) | Inconclusive | | Small cohort. Short follow-up period. |

reproduction study performed at Litton-Bionetics by Beliles et al. (1980); three studies by Bio/Dynamics Inc. (1980a, b, c) in which acrylonitrile was administered in drinking water and via gastric intubation; and an inhalation study by Quast et al. (1980b).

A second inhalation study by Maltoni et al. (1977) exposed rats to atmosphere containing 5, 10, 20, and 40 ppm acrylonitrile 4 hours per day, 5 days per week, for 12 months. Marginal increases in tumors of the mammary gland in females and the forestomach in males were observed, although the sensitivity of this test was limited by the relatively low dose levels and the short duration of exposure.

Ten epidemiologic studies of acrylonitrile and cancer have been reported (Table 13-39): five published [Monson (1978), O'Berg (1980), Thiess et al. (1980), Werner and Carter (1981), Delzell and Monson (1982)] and five unpublished [Gaffey and Strauss (1981), Herman (1981), Kiesselbach et al. (1980), Stallard (1982), and Zack (1980)]. Six of these studies present no evidence of carcinogenic risk from exposure to acrylonitrile. However, all suffer from problems in the design or methodology, i.e., small cohort size, insufficient characterization of exposure, short follow-up, and relatively youthful cohort. Because of these problems, none of these studies can be cited as adequate evidence that acrylonitrile is not carcinogenic.

Data presented in the remaining four epidemiologic studies consistently demonstrate a statistically significant risk of lung cancer in various subgroups of the populations studied. All four have problems with the methodology, definition and/or size of the population, whether exposure to other carcinogens occurred, and short follow-up intervals. In three of the four studies [Delzell and Monson (1982), Thiess et al. (1980), Werner and Carter (1981)], the problems were sufficient to cast doubt on the finding of significantly elevated risks of lung cancer found in each study. In the fourth study by O'Berg, the problems were insufficient to obscure the significant finding of lung cancer. After adjusting for latent factors and evaluating the contribution due to smoking, the finding of a statistically significantly elevated risk of lung cancer remained. Thus, one study appears adequate and three are suggestive, while the remaining six are inadequate to address the issue of a risk of lung cancer.

In addition to lung cancer, two other findings of concern are the significantly elevated risk of lymph system cancer found in the Thiess et al. study (4 observed versus 1.38 expected, $P < 0.05$) and the significantly elevated risk of stomach cancer found in the Werner and Carter study (5 observed versus 1.9 expected, $P < 0.05$). These findings provide additional suggestive evidence of the carcinogenicity of acrylonitrile.

This level of animal evidence would be regarded as "sufficient" evidence of carcinogenicity according to the International Agency for Research on Cancer (IARC) classification scheme. The human evidence for the carcinogenicity of acrylonitrile would be regarded as somewhere between "sufficient" and "limited" using the IARC classification. Therefore, in combining the human and animal evidence, acrylonitrile would be placed in group 2A, which IARC characterizes as "probably carcinogenic in humans, where the evidence for human carcinogenicity is almost sufficient."

### 13.5.4.2 Quantitative Assessment

Three unit risk estimates for air are calculated; one based on a human occupational study (O'Berg, 1980) and two based on rat cancer bioassays (Quast et al., 1980a, b). The upper-bound lifetime risk of cancer associated with a lifetime inhalation exposure of 1 $ug/m^3$ is $6.8 \times 10^{-5}$ from the human study and 1.5 x $10^{-5}$ from the rat inhalation study. The value based on the rat drinking water study is 1.5 x $10^{-4}$ (or 2.6 x $10^{-5}$ if the equivalent human dose is assumed to be mg/kg/day rather than surface area), but this study is less reliable because of the inappropriate route of exposure.

The estimate based on the human study is uncertain because of the relatively weak documentation of the available exposure estimates of the acrylonitrile workers. The air concentration had not been measured when the workers experienced their heaviest exposure and was estimated 12 years after the end of the exposure period. However, in spite of these difficulties, the estimates are consistent with those of the animal studies.

The upper-bound risk estimate for 1 ug/liter of acrylonitrile in drinking water is 1.5 x $10^{-5}$, based on the mean value of three drinking water studies in male rats. The estimate based on the mean of three female rat studies is nearly the same.

These values are regarded as rough but plausible estimates of an upper-bound of risk; i.e., it is not likely that the true risk would be much more than these values, but it could very well be considerably lower.

### 13.5.5 Conclusions

There is evidence that acrylonitrile is a human carcinogen. This conclusion is based on 1) findings of three positive drinking water rat bioassays and one positive rat gastric intubation study; 2) statistically significant positive findings of respiratory cancer in four epidemiologic studies; 3) the positive mutagenic evidence in bacteria and sister chromatid exchange tests; 4) *in vitro* evidence of interaction of acrylonitrile and/or its metabolites with DNA; and 5) acrylonitrile's structural similarity to vinyl chloride, a known animal and human carcinogen.

The carcinogenic potency of acrylonitrile is in the third quartile among 54 compounds evaluated by the Carcinogen Assessment Group as suspect carcinogens.

Using the International Agency for Research on Cancer (IARC) classification scheme, this level of evidence in animals and humans would be considered sufficient for concluding that acrylonitrile is likely to be a human carcinogen with rank of 2A.

### 13.5.6 Appendix--Comparison of Results by Various Extrapolation Models

The estimates of unit risk from animals presented in the body of this document are all calculated by the use of the linearized multistage model. The reasons for its use have been detailed therein. Essentially, it is part of a methodology that estimates a conservative linear slope at low extrapolation doses and is consistent with the data at all dose levels of the experiment. It is a nonthreshold model holding that the upper-limit of risk predicted by a linear extrapolation to low levels of the dose-response relationship is the most plausible upper-limit for the risk.

Other models have also been used for risk extrapolation. Three nonthreshold models are presented here: the one-hit, the log-Probit, and the Weibull. The one-hit model is characterized by a continuous downward curvature but is linear at low doses. It can be considered the linear form or first stage of the multistage model because of its functional form. Because of this and its downward curvature, it will always yield estimates of low level risk which are at least as large as those of the multistage model. Further, whenever the data can be fit adequately by the one-hit model, estimates from the two procedures will be comparable.

The other two models, the log-Probit and the Weibull, are often used to fit toxicological data in the observable range, because of the general "S" curvature. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-Probit model was originally proposed for use in problems of biological assay such as the assessment of potency of toxicants and drugs and has usually been used to estimate such values as percentile lethal dose or percentile effective dose. Its development was strictly empirical, i.e., it was observed that several log dose-response relationships followed the cumulative normal probability distribution function. In fitting the cancer bioassay data, assuming an independent background, this becomes:

$$P(D;a,b,c) = c + (1-c)\ \Phi\ (a+b\log_{10} D) \quad a,b > 0 \leq c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individual tolerances, and b is an estimate of the log dose-Probit response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single noncarcinogenic cell into a carcinogenic one. It has the probability distribution function:

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates. The estimate a represents the background or zero dose rate, and the parameter estimated by b represents the linear component or slope of the dose-response model. In discussing the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1-\exp-(bd^{k}) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, this model yields low-dose estimates of risks usually significantly lower than either the multistage or one-hit models, which are linear at low doses. All three of these models usually project risk estimates significantly higher at the low exposure levels than those from the log-Probit.

The estimates of added risk for low doses for the above models are given below for the various acrylonitrile data sets. Both maximum likelihood estimates and 95% upper confidence limits are presented. All estimates incorporate Abbott's correction for independent background rate.

The results (Tables 13-40, 13-41, 13-42, and 13-43) show that the maximum likelihood estimates of risk for the log-Probit model are all less than those for the other models. The one-hit model yields estimates identical with those of the multistage in six of the eight data sets and higher in the other two. The Weibull model yields risk estimates higher than those of the one-hit for five of the eight data sets. Based on past experience, this last result is unlikely. The Carcinogen Assessment Group feels that estimates based on the linearized multistage model represent the plausible upper-limits of risk.

TABLE 13-40. ESTIMATES OF LOW-DOSE RISK FROM BOTH MALE AND FEMALE SPRAGUE-DAWLEY RATS FROM THE DOW CHEMICAL COMPANY (Quast *et al.*, 1980b) INHALATION STUDY DERIVED FROM FOUR DIFFERENT MODELS (All estimates incorporate Abbott's correction for independent background rate.)

| | Maximum Likelihood Estimates of Additional Risks | | | | 95% Upper Confidence Limit of Additional Risks | | | |
|---|---|---|---|---|---|---|---|---|
| Dose ug/m$^3$ | Multistage Model | One-Hit Model | Weibull Model | Log-Probit Model | Multistage/ Model | One-Hit Model | Weibull Model | Log-Probit Model |
| **Males** | | | | | | | | |
| 0.1 ug/m$^3$ | 1.1x10$^{-7}$ | 1.6x10$^{-6}$ | 2.6x10$^{-11}$ | 0 | 1.2x10$^{-6}$ | 2.1x10$^{-6}$ | 3.9x10$^{-10}$ | 0 |
| 1 ug/m$^3$ | 1.1x10$^{-6}$ | 1.6x10$^{-5}$ | 2.0x10$^{-9}$ | 0 | 1.2x10$^{-5}$ | 2.1x10$^{-5}$ | 2.4x10$^{-8}$ | 1.9x10$^{-30}$ |
| 10 ug/m$^3$ | 1.1x10$^{-5}$ | 1.6x10$^{-4}$ | 1.6x10$^{-7}$ | 0 | 1.2x10$^{-4}$ | 2.1x10$^{-4}$ | 1.5x10$^{-6}$ | 2.0x10$^{-19}$ |
| **Females** | | | | | | | | |
| 0.1 ug/m$^3$ | 1.2x10$^{-6}$ | 1.2x10$^{-6}$ | 1.4x10$^{-6}$ | 1.1x10$^{-16}$ | 1.5x10$^{-6}$ | | 9.0x10$^{-6}$ | 3.1x10$^{-15}$ |
| 1 ug/m$^3$ | 1.2x10$^{-5}$ | 1.2x10$^{-5}$ | 1.3x10$^{-5}$ | 5.0x10$^{-12}$ | 1.5x10$^{-5}$ | | 7.4x10$^{-5}$ | 9.5x10$^{-10}$ |
| 10 ug/m$^3$ | 1.2x10$^{-4}$ | 1.2x10$^{-4}$ | 1.3x10$^{-4}$ | 3.3x10$^{-8}$ | 1.5x10$^{-4}$ | | 5.8x10$^{-4}$ | 3.9x10$^{-6}$ |

TABLE 13-41. ESTIMATES OF LOW-DOSE RISK FROM BOTH MALE AND FEMALE SPRAGUE-DAWLEY RATS FROM THE BIO/DYNAMICS INC. (1980a) DRINKING WATER STUDY DERIVED FROM FOUR DIFFERENT MODELS (All estimates incorporate Abbott's correction for independent background rate.)

| | Maximum Likelihood Estimates of Additional Risks | | | | 95% Upper Confidence Limit of Additional Risks | | |
|---|---|---|---|---|---|---|---|
| Dose ug/l | Multistage Model | One-Hit Model | Weibull Model | Log-Probit Model | Multistage/One-Hit Model Model | Weibull Model | Log-Probit Model |
| **Males** | | | | | | | |
| 0.1 ug/l | $2.4x10^{-7}$ | $8.4x10^{-7}$ | $2.4x10^{-8}$ | $1.7x10^{-15}$ | $1.1x10^{-6}$ | $3.3x10^{-5}$ | $5.2x10^{-13}$ |
| 1 ug/l | $2.4x10^{-6}$ | $8.4x10^{-6}$ | $4.7x10^{-7}$ | $2.4x10^{-11}$ | $1.1x10^{-5}$ | $5.1x10^{-5}$ | $5.5x10^{-9}$ |
| 10 ug/l | $2.4x10^{-5}$ | $8.4x10^{-5}$ | $8.6x10^{-6}$ | $7.1x10^{-8}$ | $1.1x10^{-4}$ | $7.4x10^{-4}$ | $1.0x10^{-5}$ |
| **Females** | | | | | | | |
| 0.1 ug/l | $8.1x10^{-7}$ | $8.1x10^{-7}$ | $1.8x10^{-4}$ | $3.0x10^{-7}$ | $1.1x10^{-6}$ | $7.4x10^{-4}$ | $3.5x10^{-6}$ |
| 1 ug/l | $8.1x10^{-6}$ | $8.1x10^{-6}$ | $7.1x10^{-4}$ | $1.5x10^{-5}$ | $1.1x10^{-5}$ | $2.5x10^{-3}$ | $1.3x10^{-4}$ |
| 10 ug/l | $8.1x10^{-5}$ | $8.1x10^{-5}$ | $2.8x10^{-3}$ | $4.0x10^{-4}$ | $1.1x10^{-4}$ | $8.5x10^{-3}$ | $2.3x10^{-3}$ |

TABLE 13-42. ESTIMATES OF LOW-DOSE RISK FROM BOTH MALE AND FEMALE SPRAGUE-DAWLEY RATS FROM THE DOW CHEMICAL COMPANY (Quast _et al._, 1980a) DRINKING WATER STUDY DERIVED FROM FOUR DIFFERENT MODELS
(All estimates incorporate Abbott's correction for independent background rate.)

| | Maximum Likelihood Estimates of Additional Risks | | | | 95% Upper Confidence Limit of Additional Risks | | |
|---|---|---|---|---|---|---|---|
| Dose ug/1 | Multistage Model | One-Hit Model | Weibull Model | Log-Probit Model | Multistage/One-Hit Model Model | Weibull Model | Log-Probit Model |
| Males | | | | | | | |
| 0.1 ug/1 | $2.3 \times 10^{-6}$ | $2.3 \times 10^{-6}$ | $2.3 \times 10^{-6}$ | 0 | $2.8 \times 10^{-6}$ | $1.2 \times 10^{-5}$ | 0 |
| 1 ug/1 | $2.3 \times 10^{-5}$ | $2.3 \times 10^{-5}$ | $2.3 \times 10^{-5}$ | 0 | $2.8 \times 10^{-5}$ | $1.0 \times 10^{-4}$ | $1.0 \times 10^{-26}$ |
| 10 ug/1 | $2.3 \times 10^{-4}$ | $2.3 \times 10^{-4}$ | $2.3 \times 10^{-4}$ | $1.1 \times 10^{-16}$ | $2.8 \times 10^{-4}$ | $8.4 \times 10^{-4}$ | $2.1 \times 10^{-15}$ |
| Females | | | | | | | |
| 0.1 ug/1 | $2.2 \times 10^{-6}$ | $2.2 \times 10^{-6}$ | $2.4 \times 10^{-5}$ | 0 | $2.6 \times 10^{-6}$ | $1.1 \times 10^{-4}$ | $1.5 \times 10^{-27}$ |
| 1 ug/1 | $2.2 \times 10^{-5}$ | $2.2 \times 10^{-5}$ | $1.6 \times 10^{-4}$ | 0 | $2.6 \times 10^{-5}$ | $6.5 \times 10^{-4}$ | $1.2 \times 10^{-18}$ |
| 10 ug/1 | $2.2 \times 10^{-4}$ | $2.2 \times 10^{-4}$ | $1.0 \times 10^{-3}$ | $8.0 \times 10^{-13}$ | $2.6 \times 10^{-4}$ | $3.6 \times 10^{-3}$ | $1.7 \times 10^{-11}$ |

TABLE 13-43. ESTIMATES OF LOW-DOSE RISK FROM BOTH MALE AND FEMALE SPRAGUE-DAWLEY RATS FROM THE BIO/DYNAMICS INC. (1980b) DRINKING WATER STUDY DERIVED FROM FOUR DIFFERENT MODELS (All estimates incorporate Abbott's correction for independent background rate.)

| | Maximum Likelihood Estimates of Additional Risks | | | | 95% Upper Confidence Limit of Additional Risks | | |
|---|---|---|---|---|---|---|---|
| Dose ug/1 | Multistage Model | One-Hit Model | Weibull Model | Log-Probit Model | Multistage/One-Hit Model | Weibull Model | Log-Probit Model |
| **Males** | | | | | | | |
| 0.1 ug/1 | $9.0 \times 10^{-7}$ | $9.0 \times 10^{-7}$ | $1.6 \times 10^{-5}$ | $2.7 \times 10^{-11}$ | $1.1 \times 10^{-6}$ | $7.0 \times 10^{-5}$ | $3.9 \times 10^{-10}$ |
| 1 ug/1 | $9.0 \times 10^{-6}$ | $9.0 \times 10^{-6}$ | $9.3 \times 10^{-5}$ | $2.1 \times 10^{-8}$ | $1.1 \times 10^{-5}$ | $3.6 \times 10^{-4}$ | $2.2 \times 10^{-7}$ |
| 10 ug/1 | $9.0 \times 10^{-5}$ | $9.0 \times 10^{-5}$ | $5.5 \times 10^{-4}$ | $5.1 \times 10^{-6}$ | $1.1 \times 10^{-4}$ | $1.8 \times 10^{-3}$ | $3.6 \times 10^{-5}$ |
| **Females** | | | | | | | |
| 0.1 ug/1 | $6.3 \times 10^{-7}$ | $6.3 \times 10^{-7}$ | $7.2 \times 10^{-5}$ | $6.3 \times 10^{-8}$ | $8.2 \times 10^{-7}$ | $2.6 \times 10^{-4}$ | $5.2 \times 10^{-7}$ |
| 1 ug/1 | $6.3 \times 10^{-6}$ | $6.3 \times 10^{-6}$ | $3.2 \times 10^{-4}$ | $3.9 \times 10^{-6}$ | $8.2 \times 10^{-6}$ | $9.6 \times 10^{-4}$ | $2.3 \times 10^{-5}$ |
| 10 ug/1 | $6.3 \times 10^{-5}$ | $6.3 \times 10^{-5}$ | $1.3 \times 10^{-3}$ | $1.3 \times 10^{-4}$ | $8.2 \times 10^{-5}$ | $3.6 \times 10^{-3}$ | $5.2 \times 10^{-4}$ |

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